Scheme 1

Solid Phase Protein Synthesis

Native Chemical Ligations in an N- to C-Terminal Direction

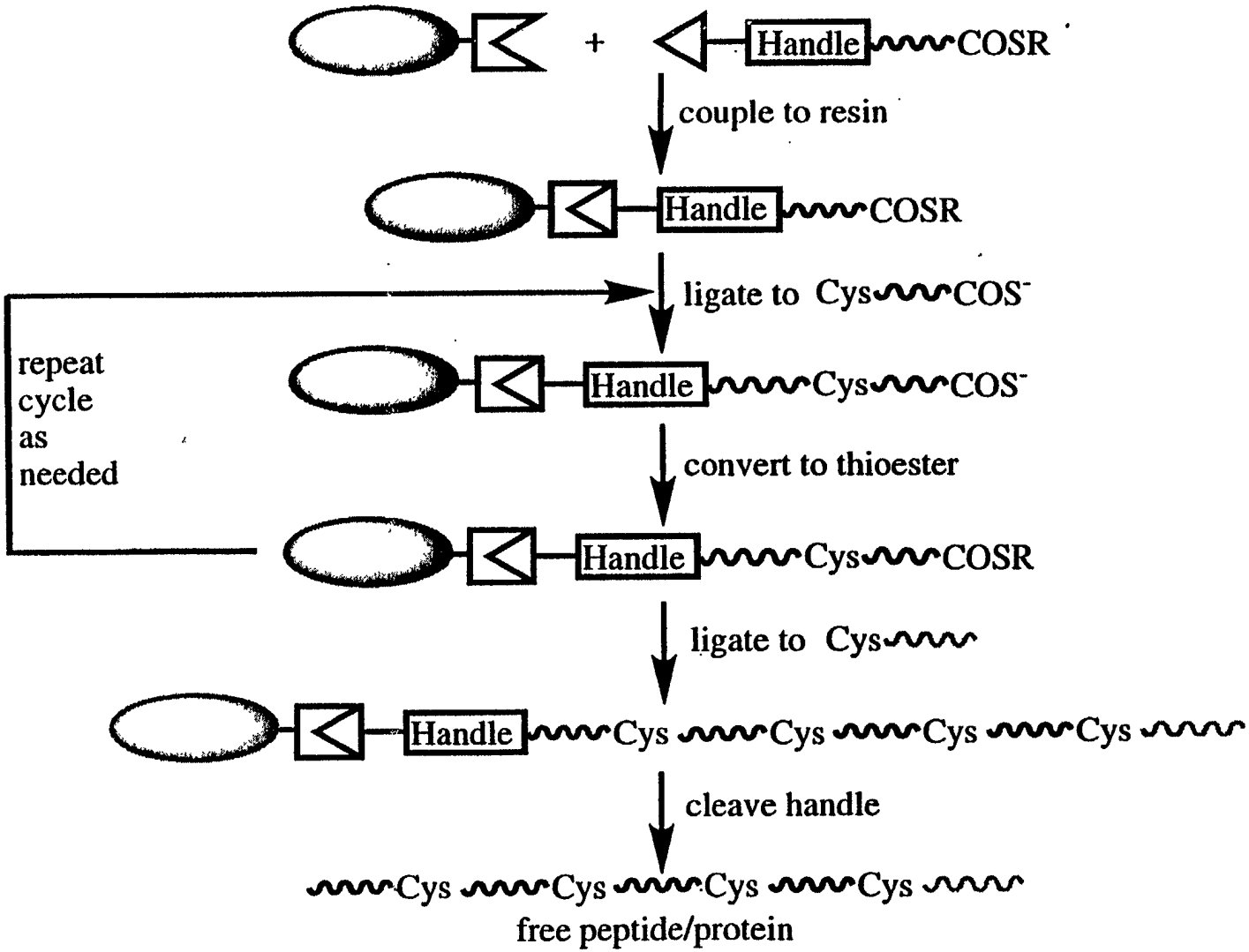


FIG. 1

Cys + COSR Stability Under Ligation Conditions

1. In the absence of a thioester peptide
   H-CGFRVREFGDNTA-*COSH* MW=1487.6
   6M GU•HCL, 0.1M NaPi, 0.5% thiophenol, room temperature, overnight

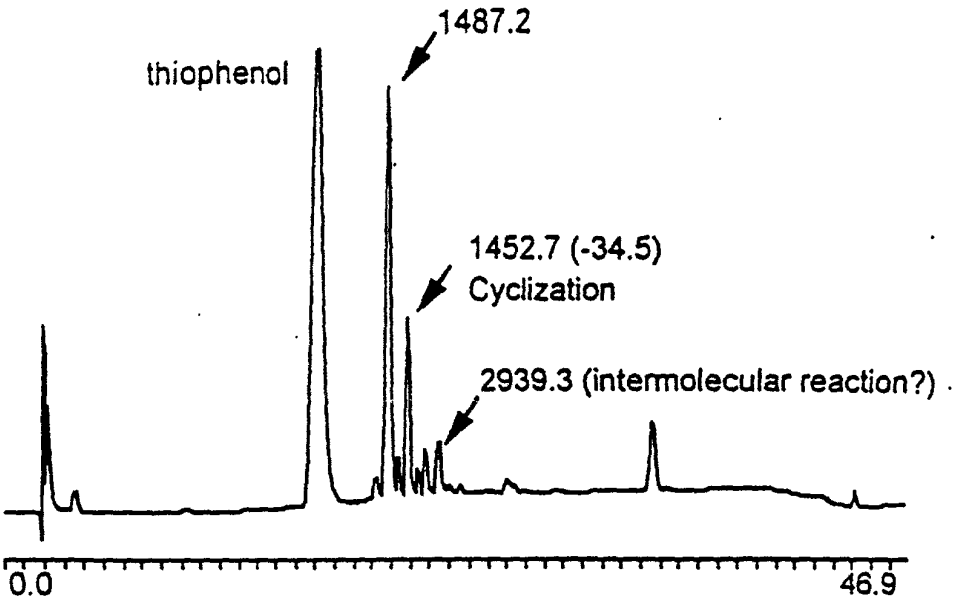


FIG. 2A

2. In the presence of a thioester peptide
   H-CGFRVREFGDNTA-*COSH* MW=1487.6 + *H*-DSVISLSGDH-*SPAL* MW= 1230.2
   MW of Ligation product = 2498.7
   6M GU•HCL, 0.1M NaPi, 0.5% thiophenol, room temperature, overnight

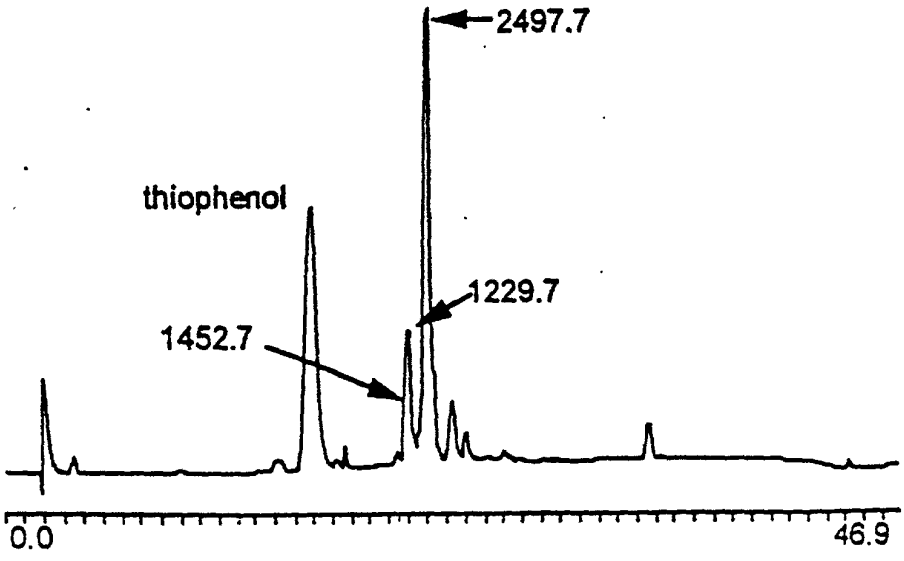


FIG. 2B

MSC Removal Experiments

MSC-CTSAGPHFNPLSRKHG-*OH* MW=1859.1
H-CTSAGPHFNPLSRKHG-*OH* MW=1708.9

Aliquot of peptide in 6M Gu•HCl, 0.1M NaPi, pH 7.5 was diluted into 1N NaOH for two minutes, quenched with 1N HCl

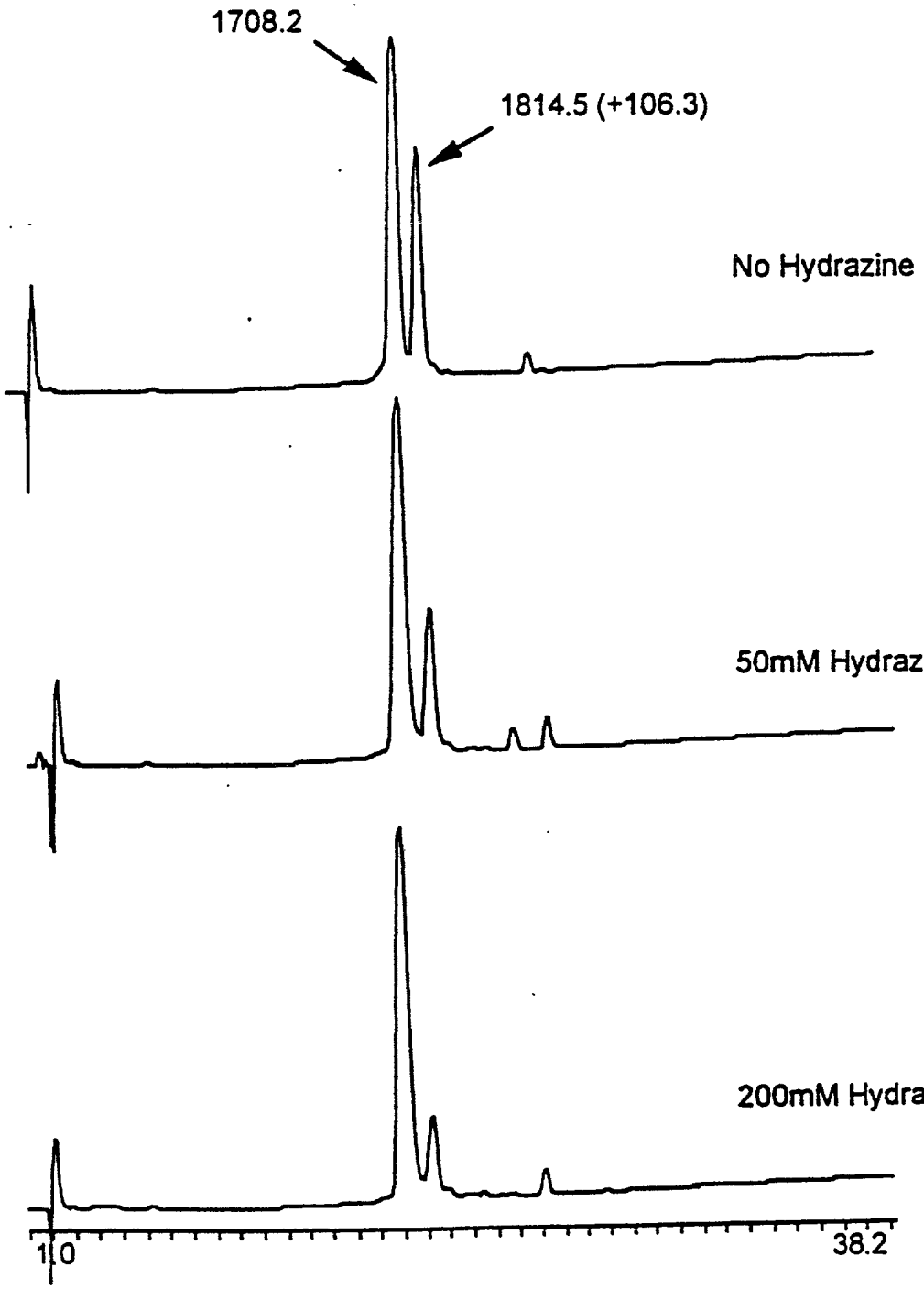


FIG. 3A

FIG. 3B

FIG. 3C

MSC Removal Experiments (Cont'd)

Lev-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH*
MW=4022.4
H-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH*
MW=3745.1

Aliquot of peptide in 6M Gu•HCl, 0.1M NaAc, pH 4.6 was diluted into 6M Gu•HCl, 0.1M NaAc, pH 14 for two minutes, quenched with 6M Gu•HCl, 0.1M NaAc, pH 2.0

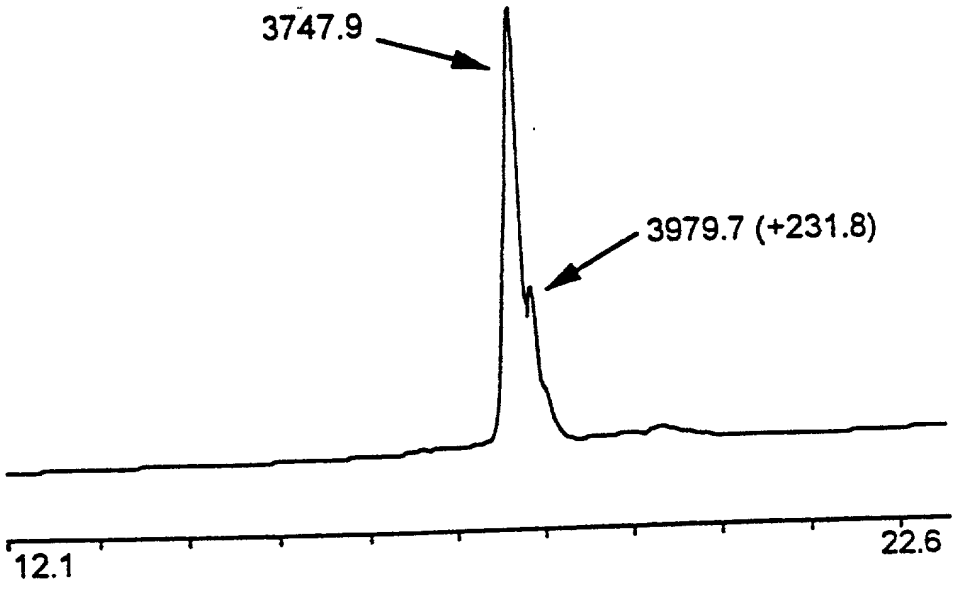


FIG. 4

Resin Preparation

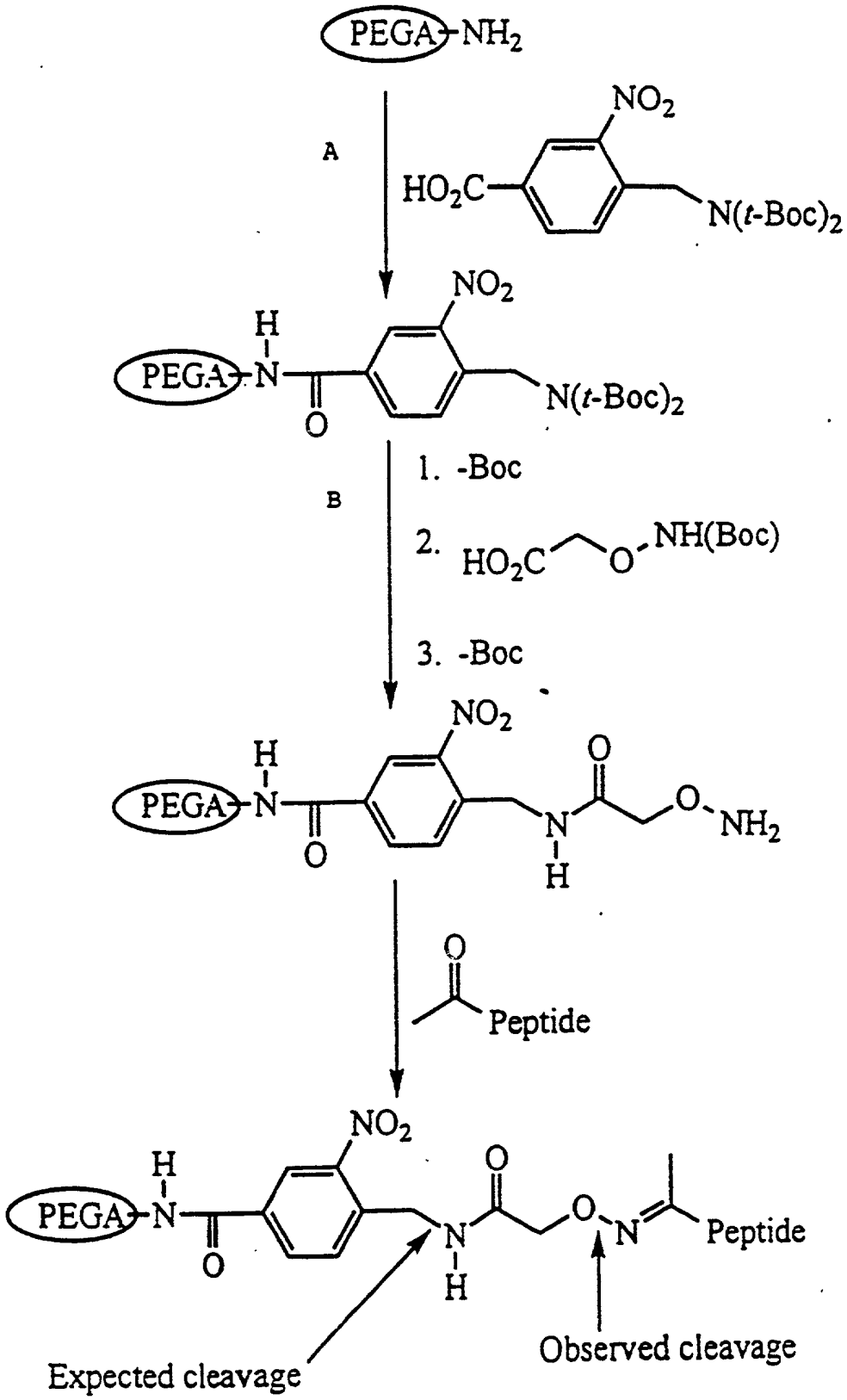
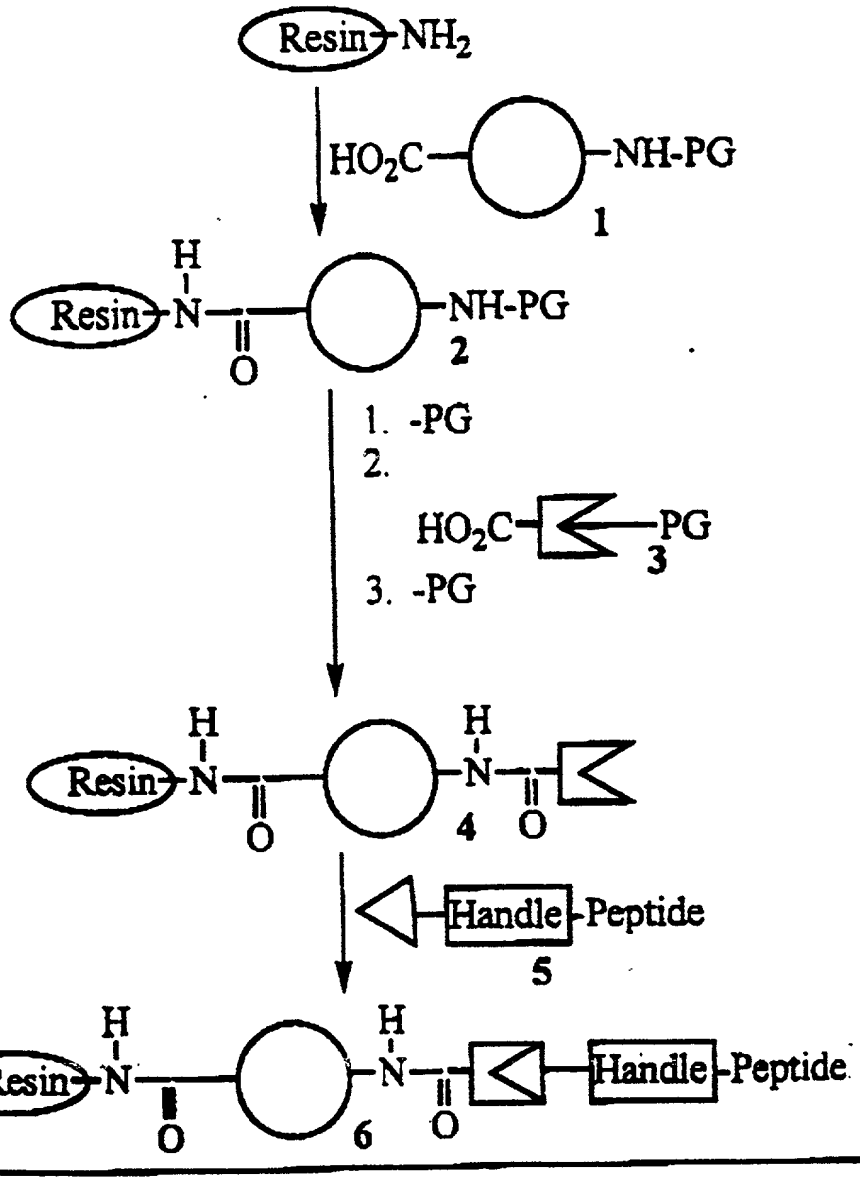


FIG. 5A

Resin Preparation



HO$_2$C-NH- = cleavable linker used for monitoring with Maldi, Electrospray Mass Spec, etc...

PG = protecting group

HO$_2$C- = functional group added to resin to couple with peptide

Handle-Peptide = peptide functionalized with 1) a cleavable handle for release of peptide/protein from the resin at completion of synthesis and 2) functional group to couple to resin

FIG. 5B

Derivatization of Segment 1 (N-terminal)

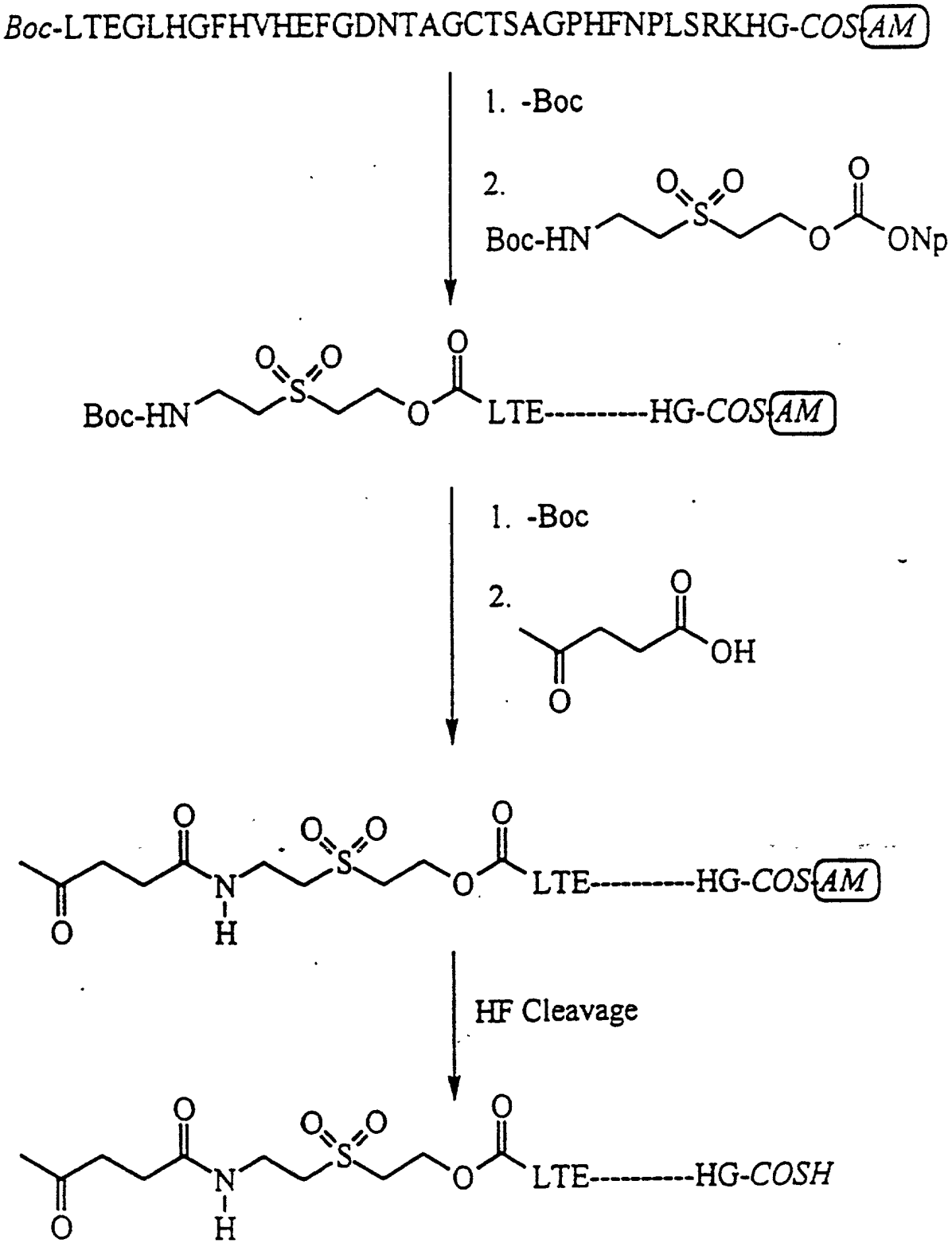


FIG. 6

Polymer-Supported Ligation on PEGA

Lev-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH* (1)
+ Resin-PCL-ONH2

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 acetate

Resin-PCL-oxime-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH* (1)

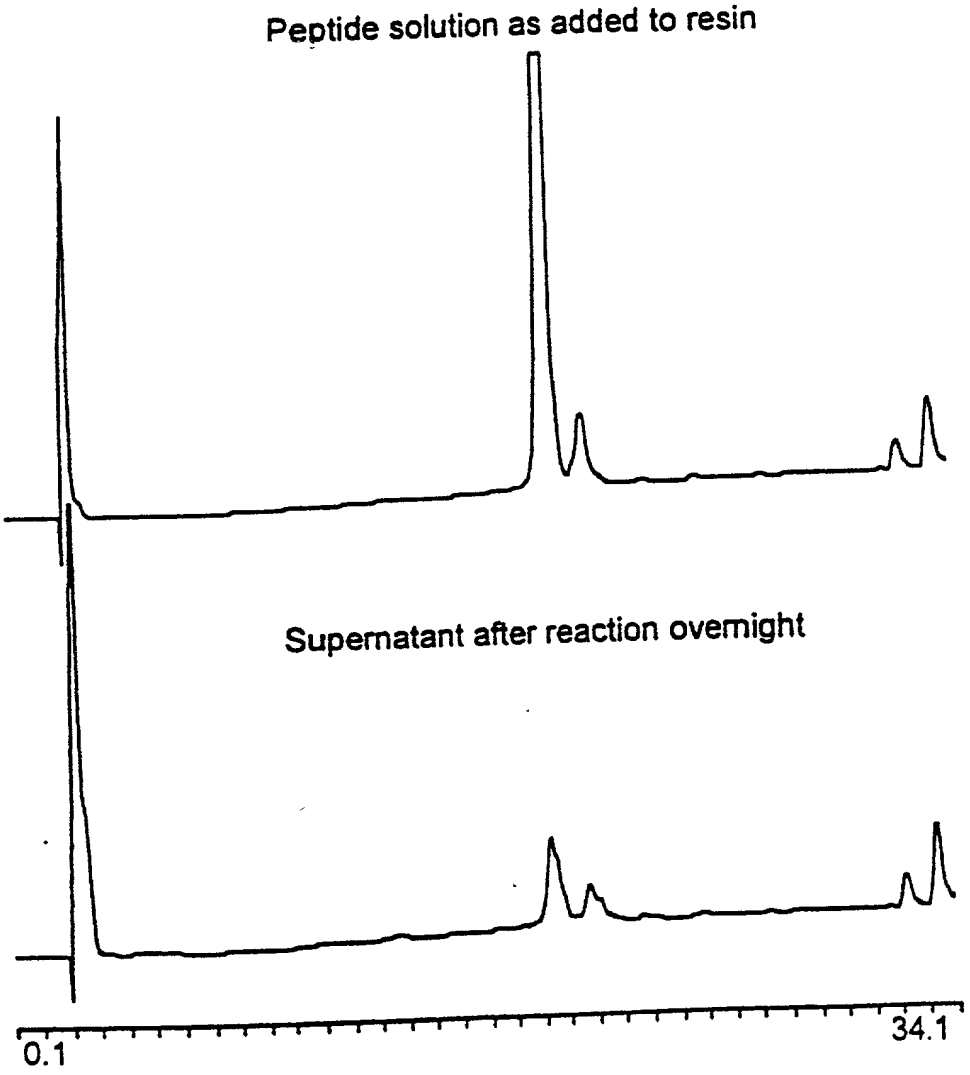


FIG. 7A

FIG. 7B

Polymer-Supported Ligation on Iseo

Lev-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH* (1)
+ Resin-PCL-ONH2

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 acetate

Resin-PCL-oxime-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH* (1)

Peptide solution as added to resin

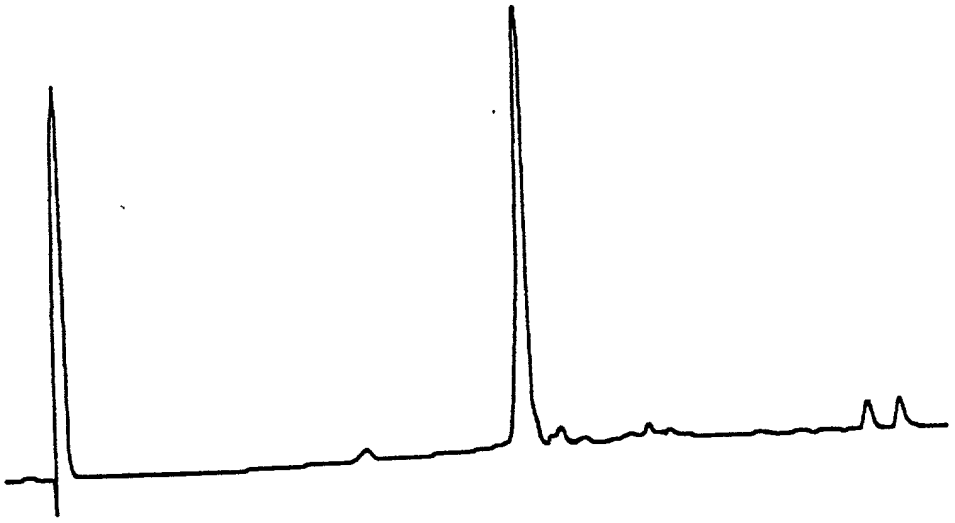


FIG. 8A

Supernatant after reaction overnight

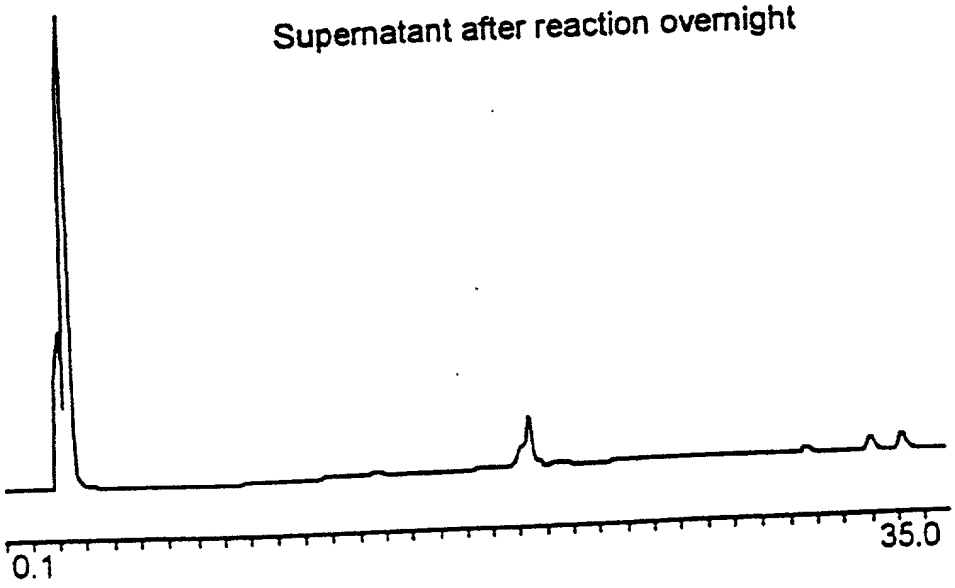


FIG. 8B

Polymer-Supported Ligation on Isco

Lev-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH* (1)
+ Resin-PCL-ONH2

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 acetate

Resin-PCL-oxime-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSH* (1)
Maldi Mass = 4022, Base Cleavage Mass = 3745

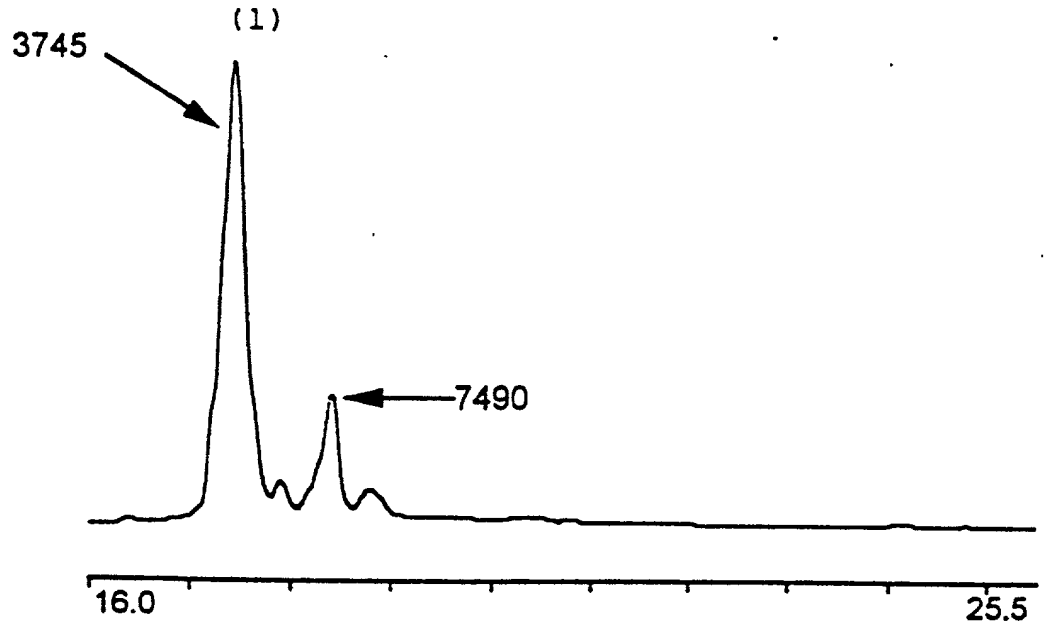


FIG. 9A

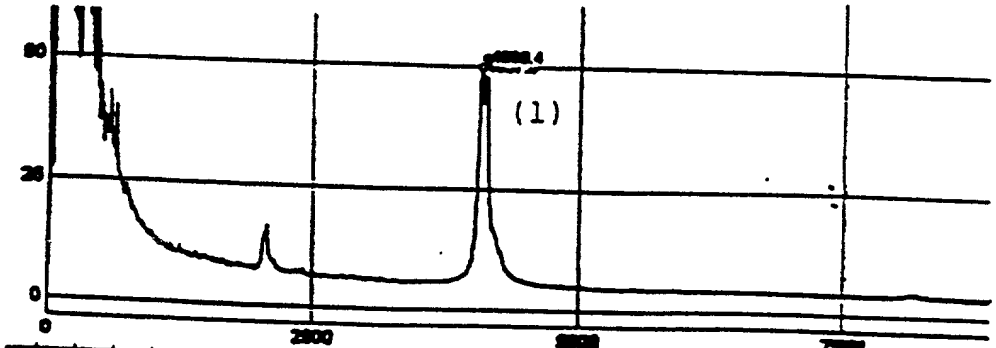


FIG. 9B

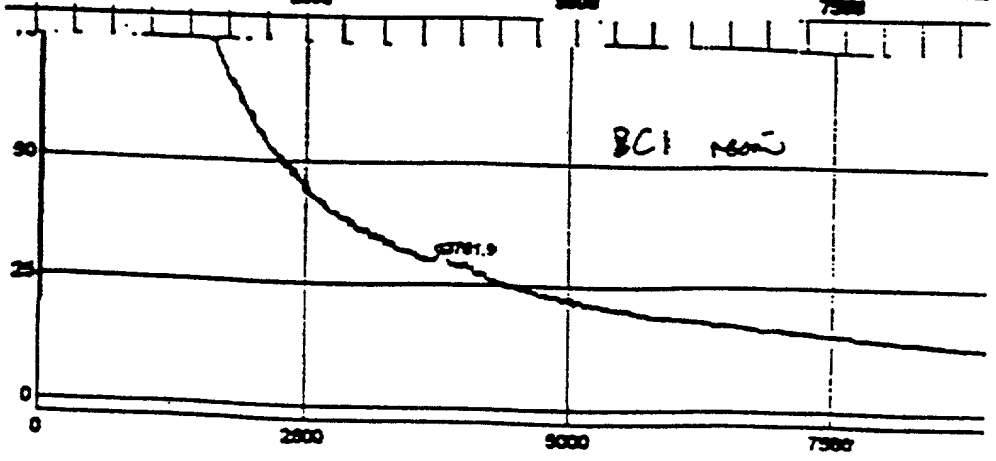


FIG. 9C

Polymer-Supported Ligation on Isco

Resin-PCL-oxime-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-*COSAc* (1)

Maldi Mass = 4080, Base Cleavage Mass = 3729

\+ *H*-CGFRVREFGDNTA-*COSH* (2)

↓ 3. pH 7.5, 6M Gu•HCl, 0.1M phosphate, 0.5% thiophenol

Resin-PCL-oxime-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHGCGFRVREF-GDNTA-*COSH* (1+2)

Maldi Mass = 5476, Base Cleavage Mass = 5199

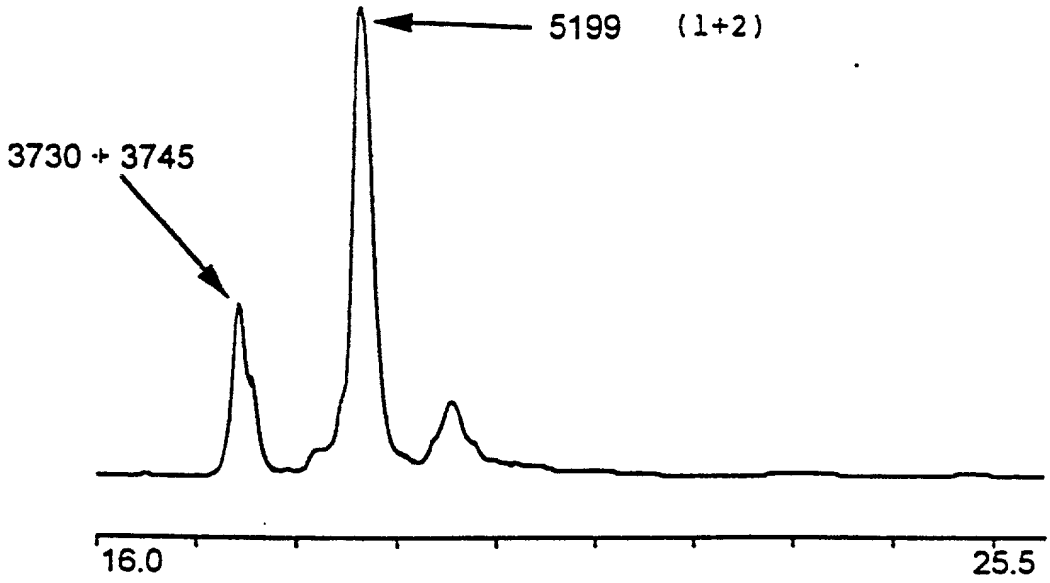


FIG. 10A

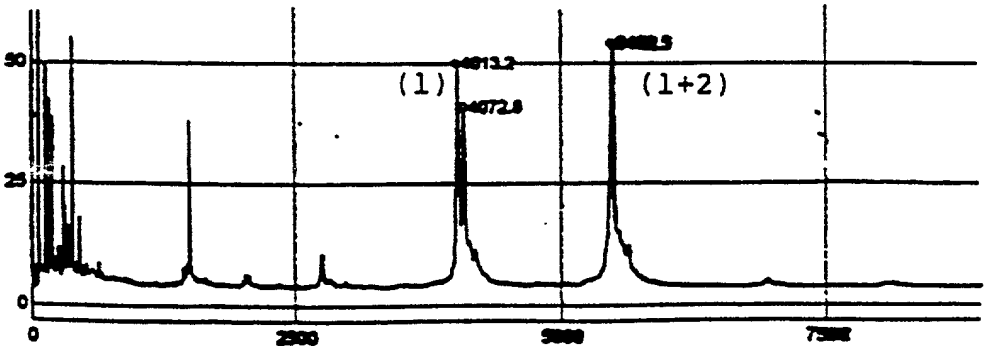


FIG. 10B

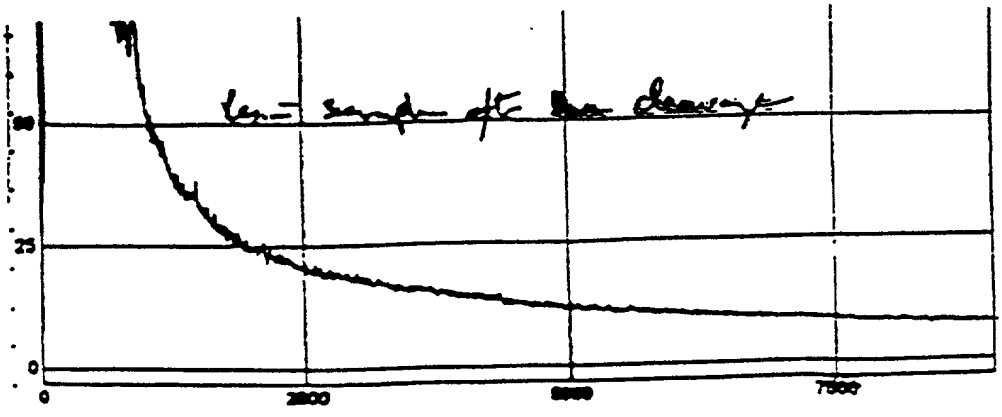


FIG. 10C

Polymer-Supported Ligation on Isco

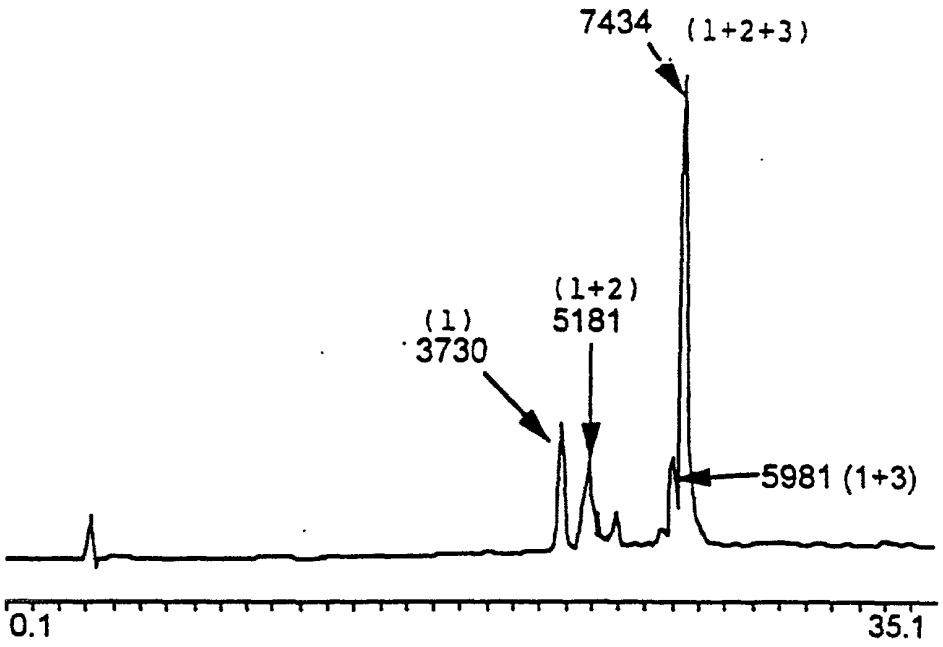


FIG. 11

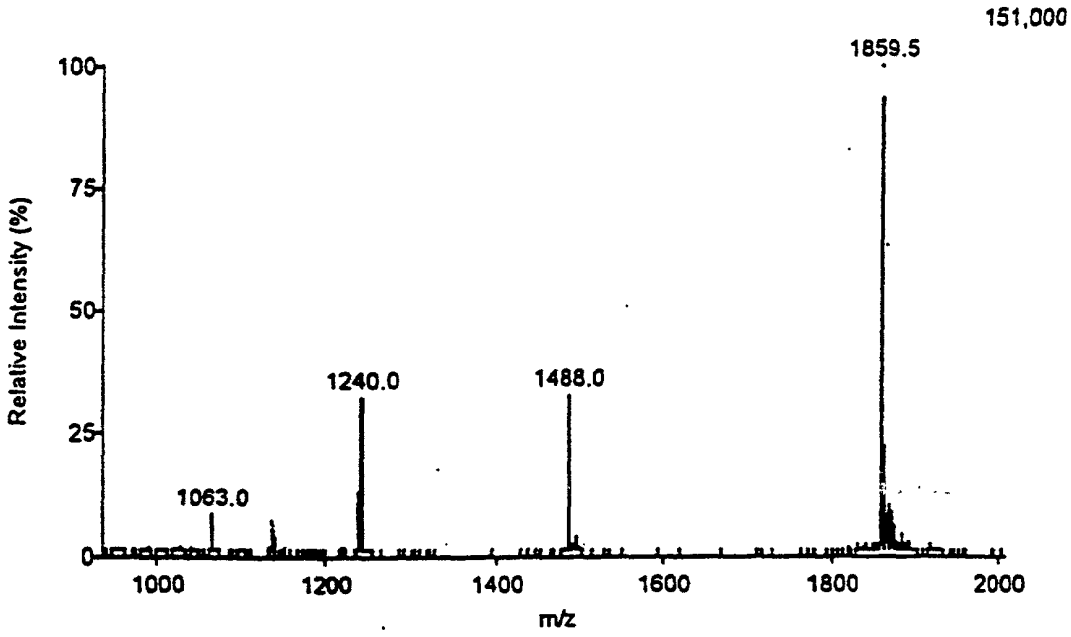


FIG. 12A

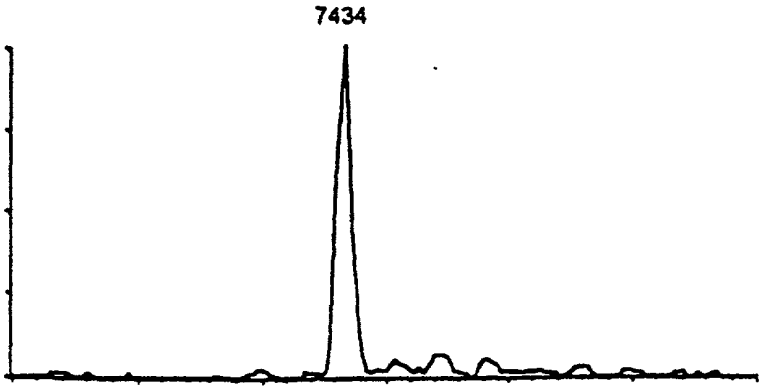


FIG. 12B

Polymer-Supported Ligation on PEGA

No photocleavable linker

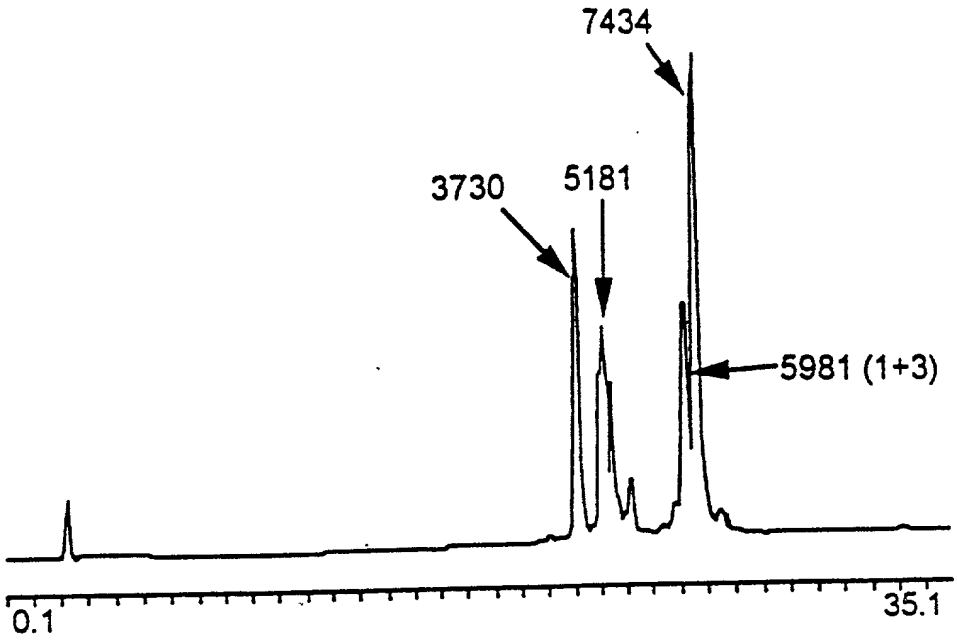


FIG. 13

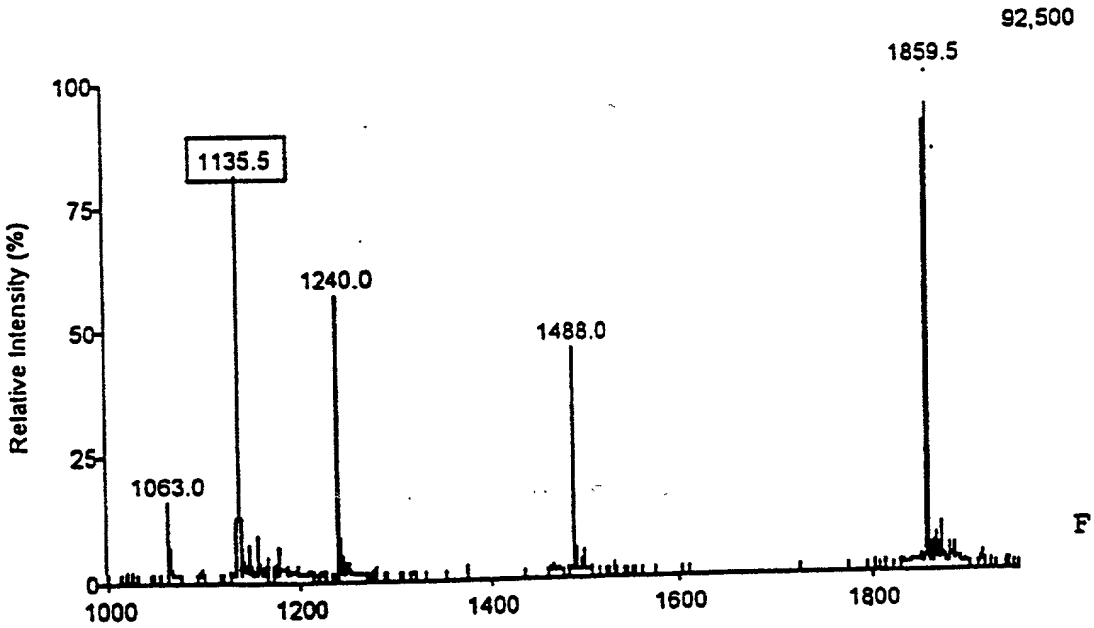


FIG. 14A

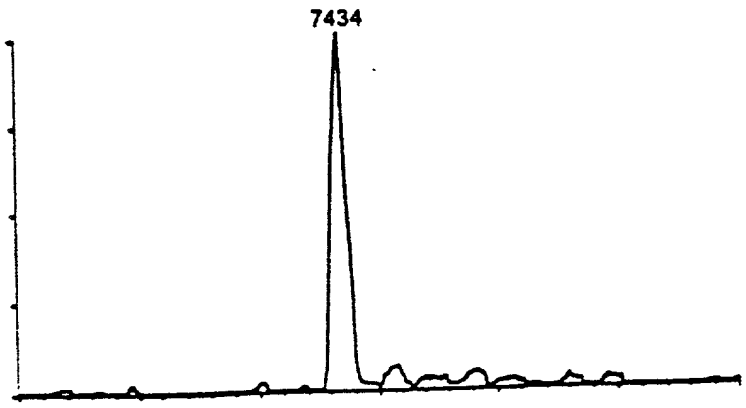


FIG. 14B

On Resin Purification

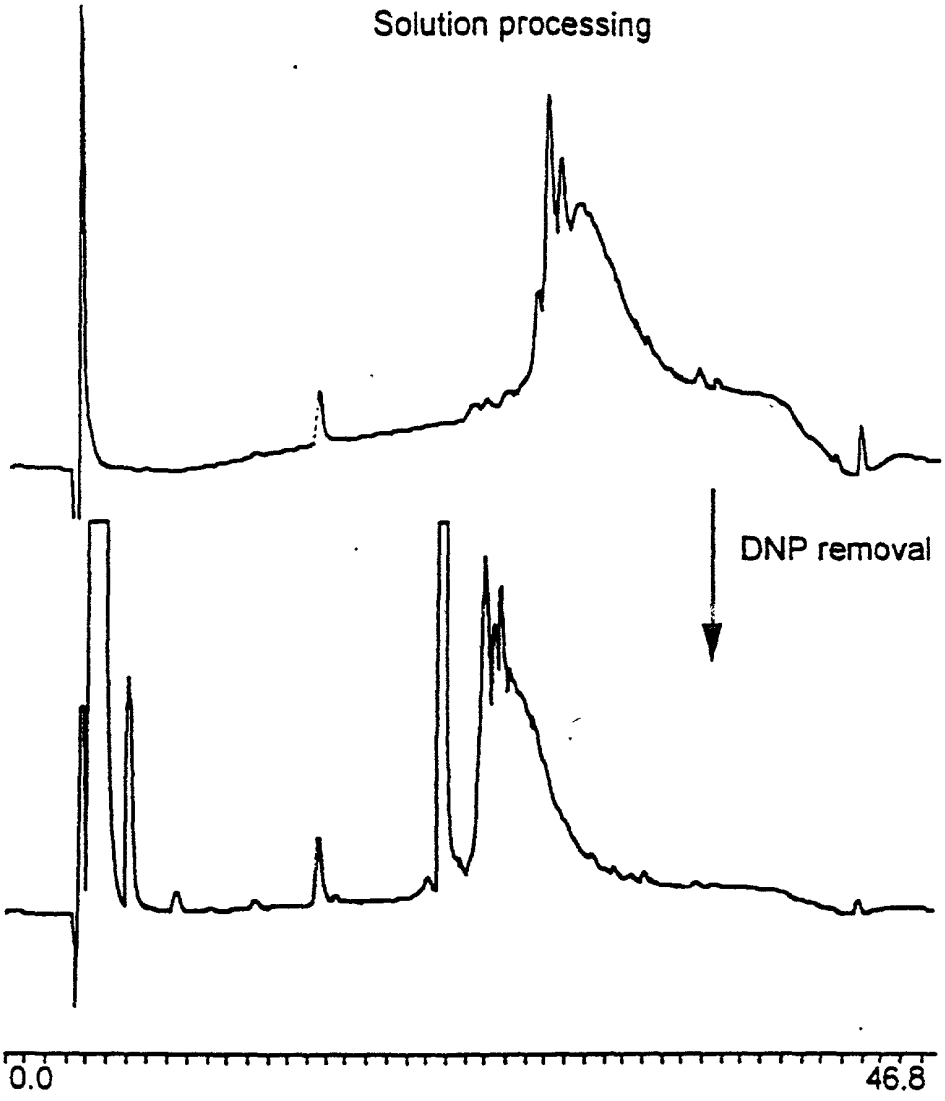


FIG. 15A

FIG. 15B

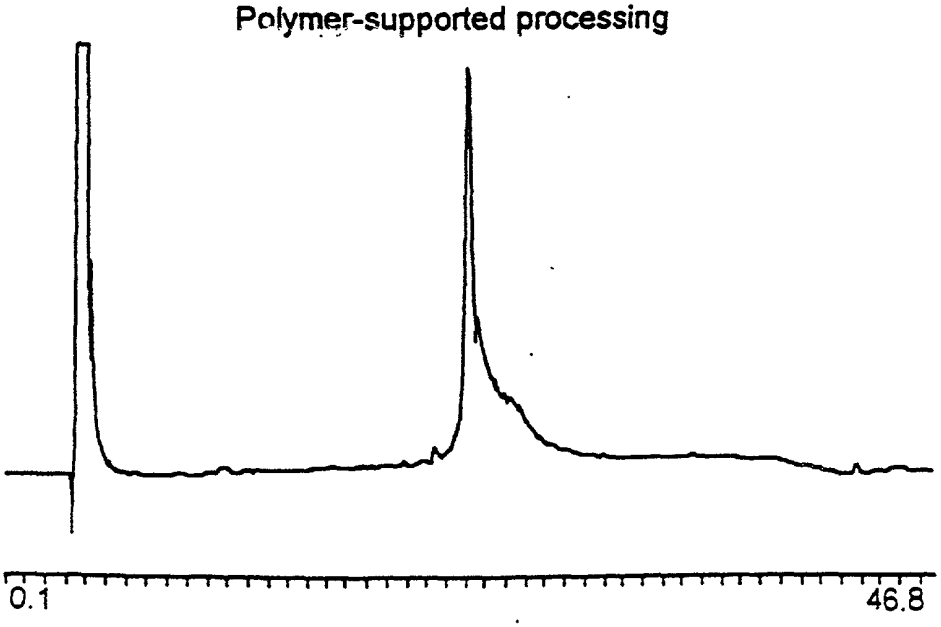


FIG. 15C

Synthesis of MIF by Solid Phase Native Ligations

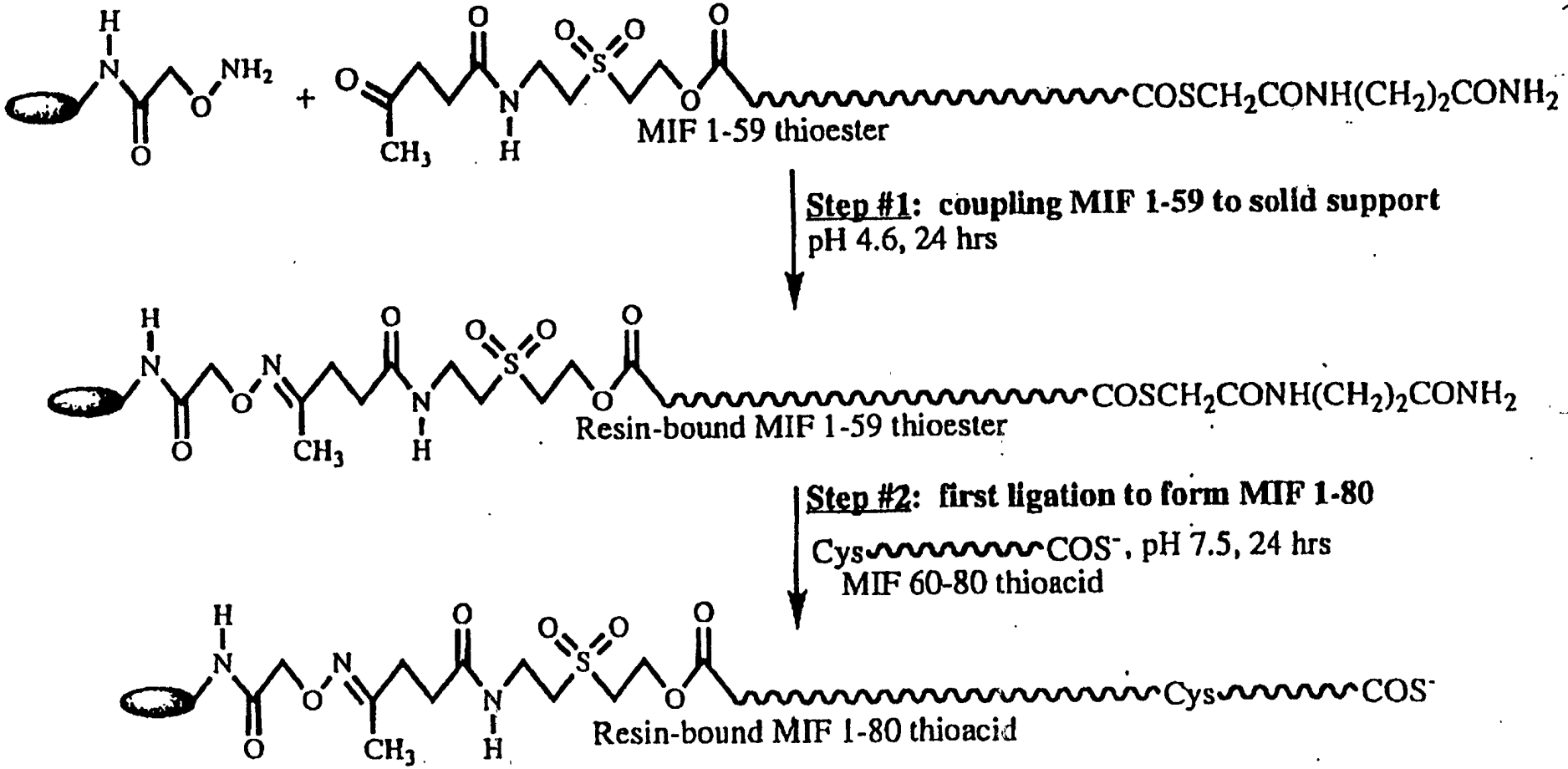


FIG. 16A

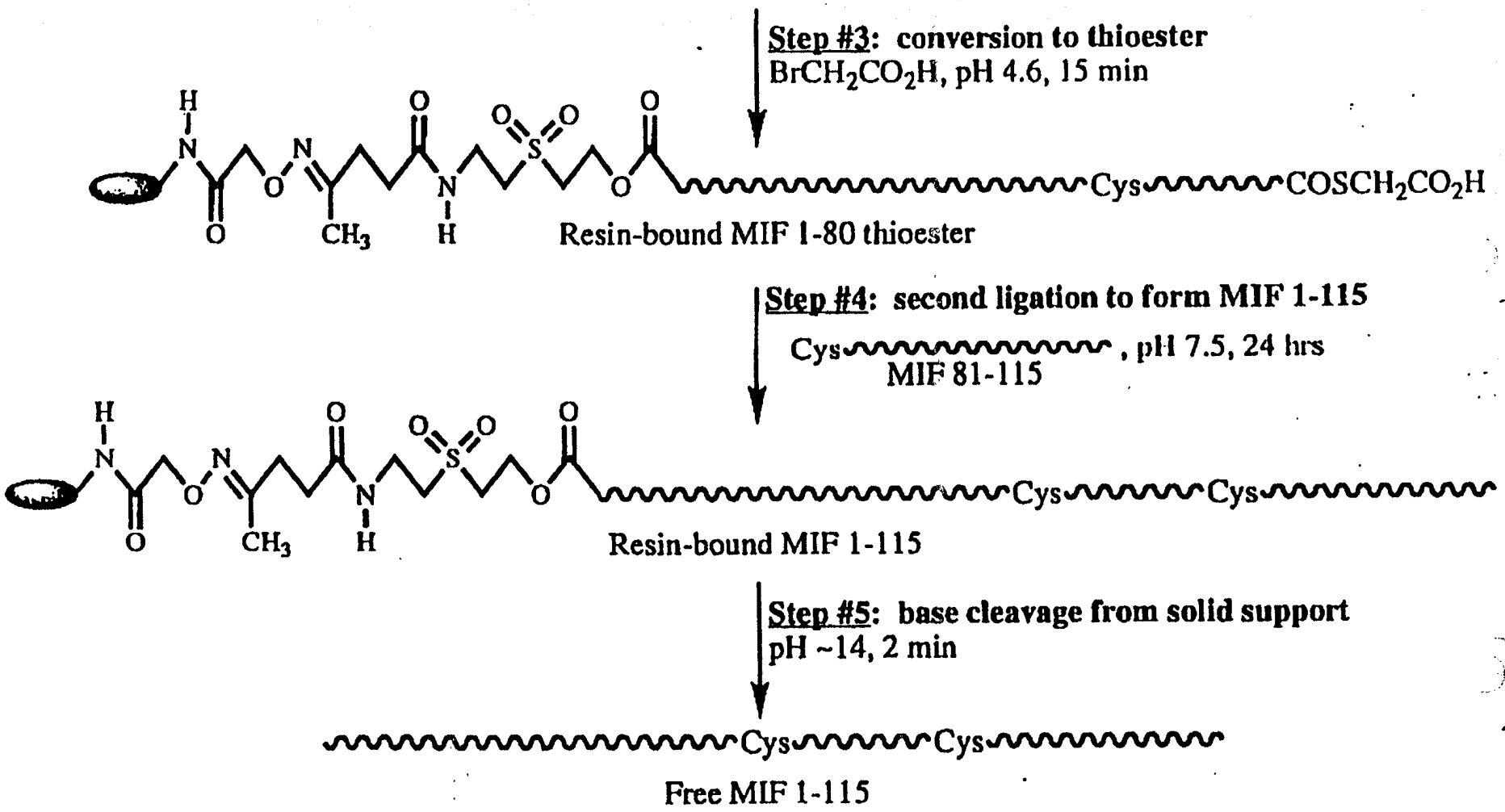

Step #3: conversion to thioester
BrCH2CO2H, pH 4.6, 15 min
Cys
COSCH2CO2H
Resin-bound MIF 1-80 thioester
Step #4: second ligation to form MIF 1-115
Cys , pH 7.5, 24 hrs
MIF 81-115
Cys
Cys
Resin-bound MIF 1-115
Step #5: base cleavage from solid support
pH ~14, 2 min
Cys
Cys
Free MIF 1-115

FIG. 16B

Modification of N-terminal Peptide Segment and Solid Support

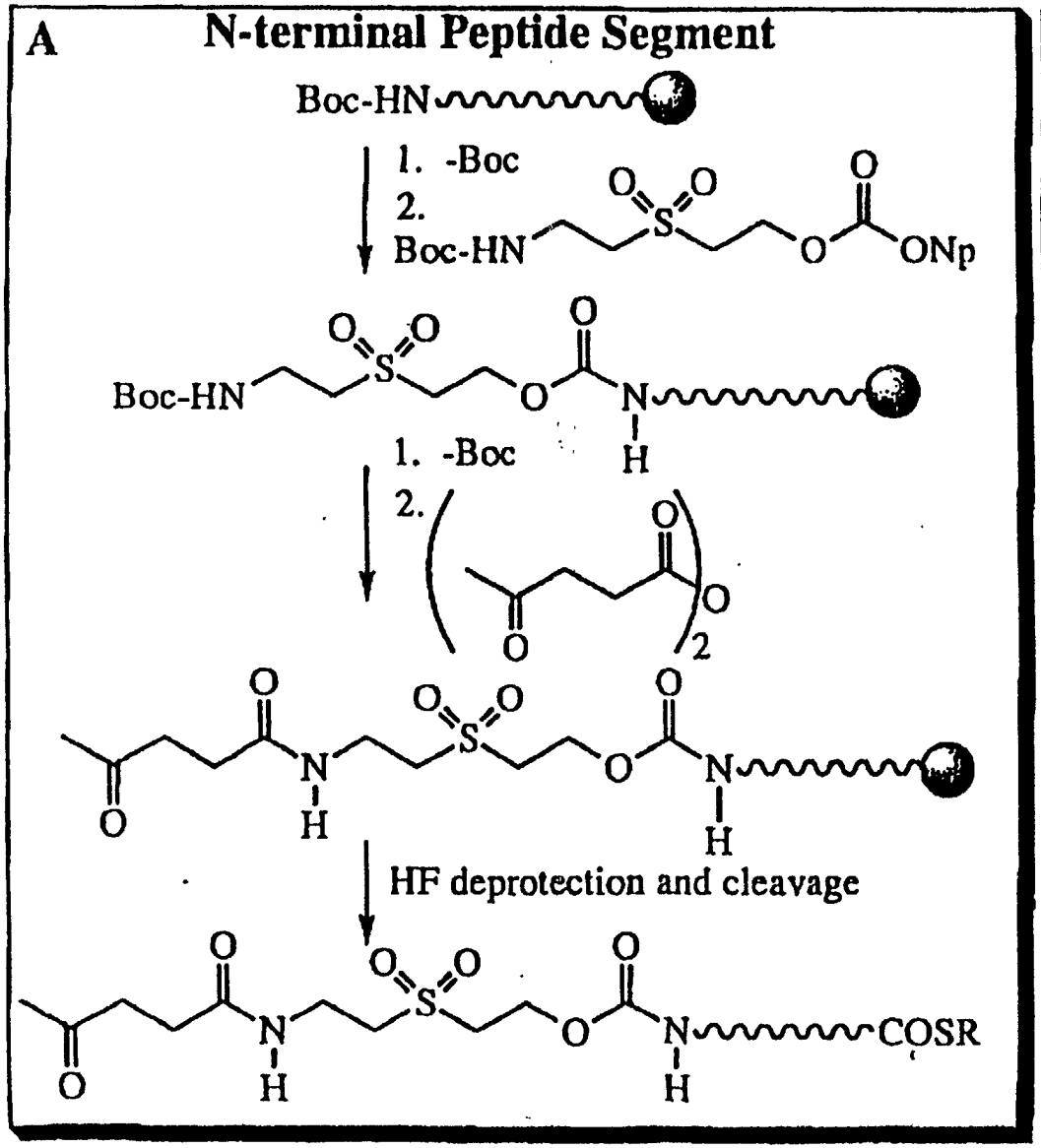


FIG 17A

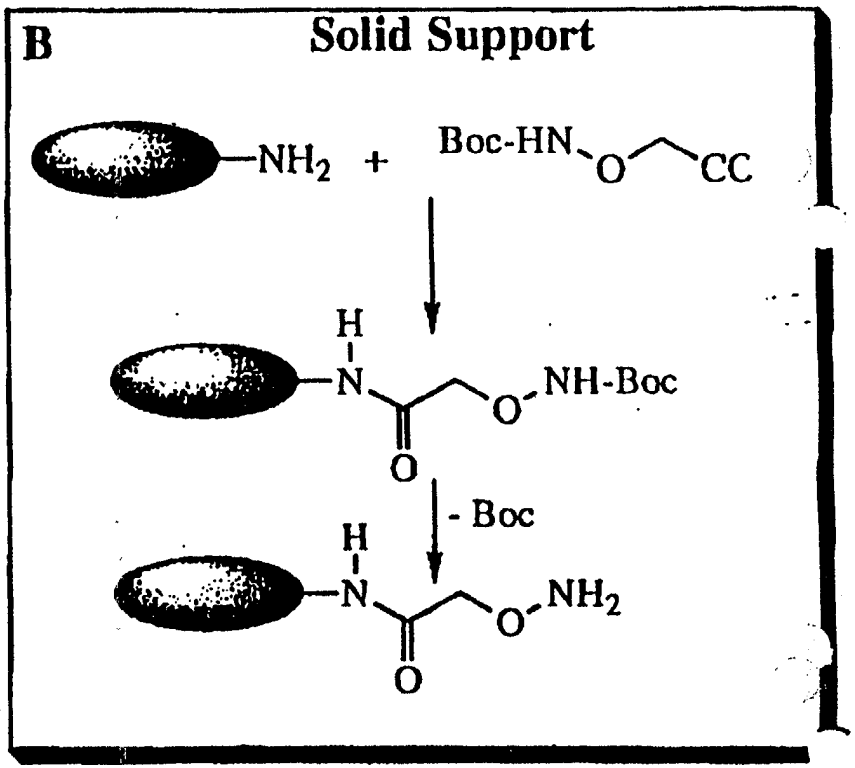
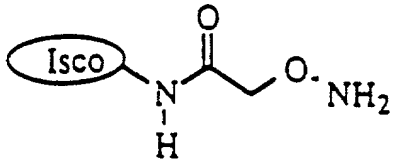


FIG. 17B

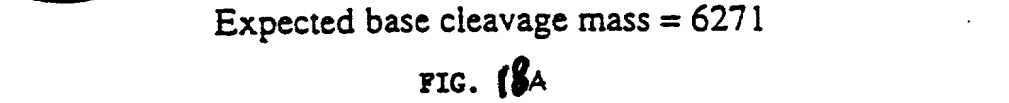
Coupling of MIF 1-59 to Solid Support



+

Ketone-MSC handle-Met^{1}-MIF 2-58-Leu^{59}-SAc-βAla-CO_2H

#1



Expected base cleavage mass = 6271

FIG. 18A

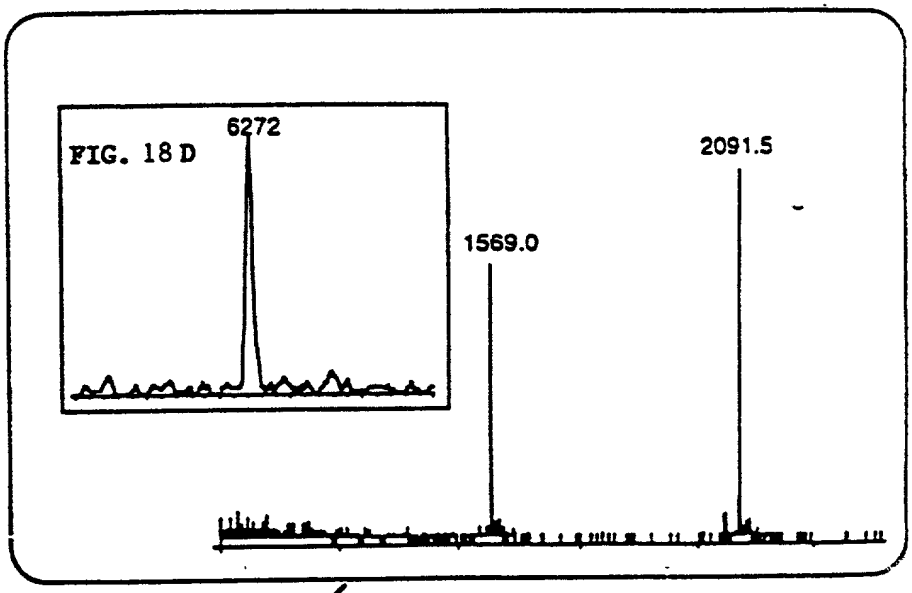


FIG. 18C

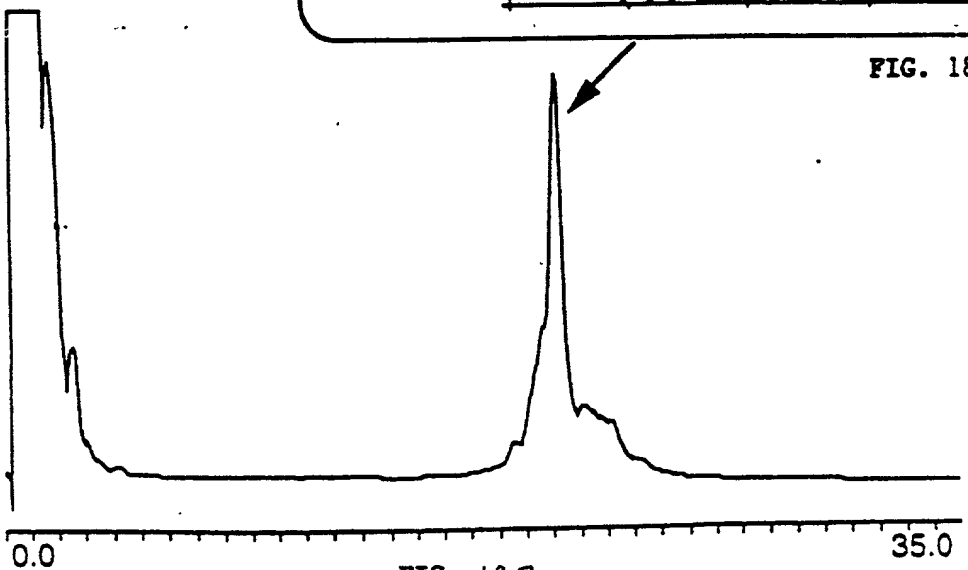


FIG. 18B

Ligation to form MIF1-80

Isco — Oxime-MSC handle-Met^{1}-MIF 2-58-Leu^{59}-SAc-βAla-CO_2H

#2 | Cys^{60}-MIF 61-79-Leu^{80}-COSH

↓

Isco — Oxime-MSC handle-Met^{1}-MIF 2-79-Leu^{80}-COSH

Expected base cleavage mass = 8502

FIG. 19A

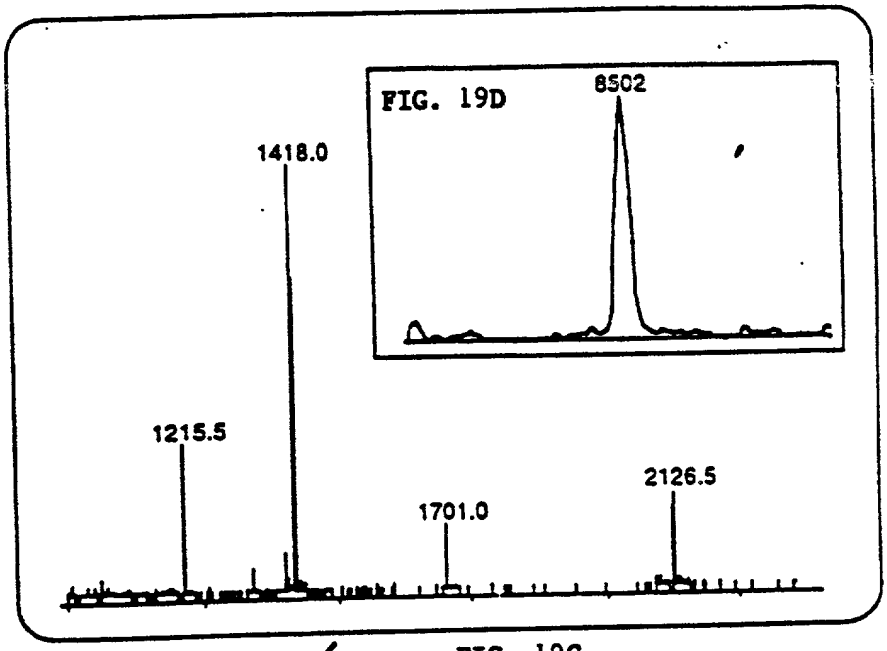


FIG. 19C

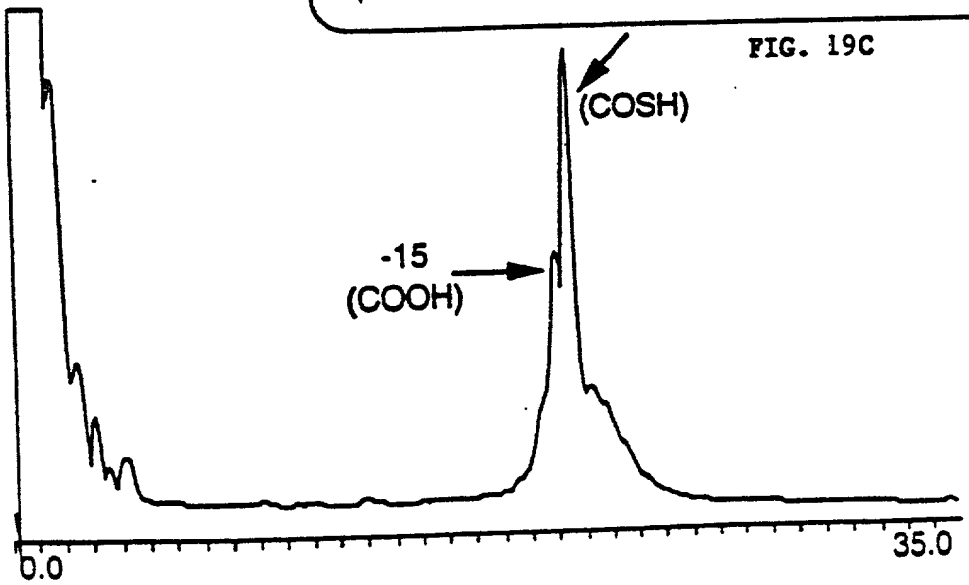


FIG. 19B

Ligation to form MIF 1-115

Isco — Oxime-MSC handle-Met[1]-MIF 2-79-Leu[80]-COSAc

#4 ↓ Cys[81]-MIF 82-114-Ala[115]-CO_2H
6M Gu•HCl, 0.1 M Na Pi, 0.5% thiophenol
0.15 M Methionine, pH 7.5

Isco — Oxime-MSC handle-Met[1]-MIF 2-114-Ala[115]-CO_2H
Expected base cleavage mass = 12450

FIG. 20A

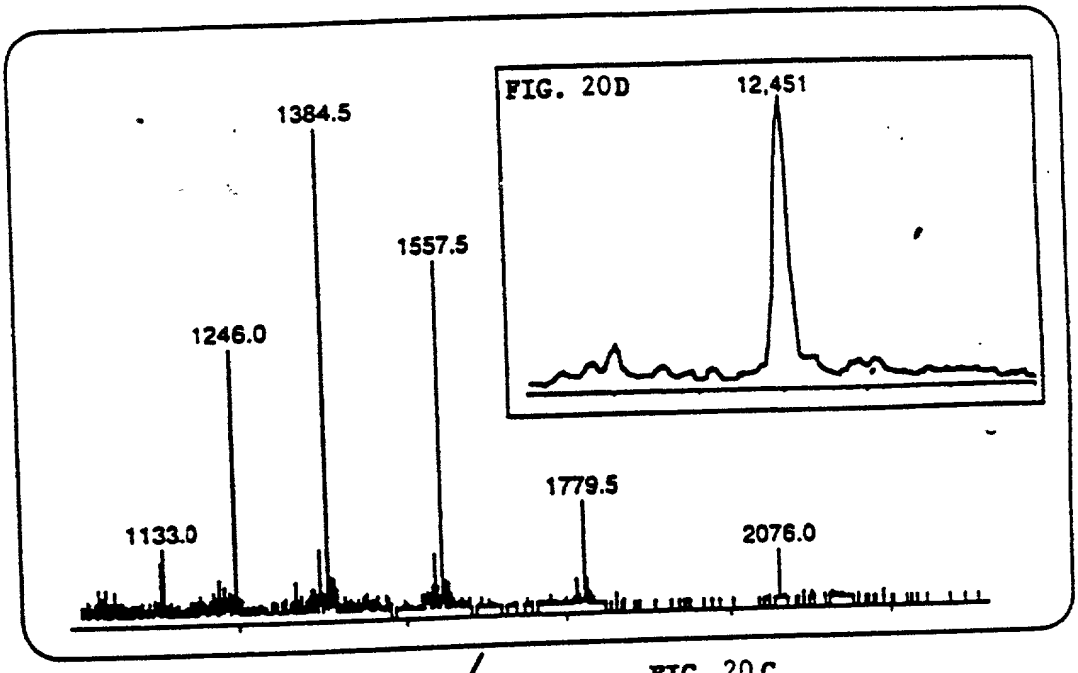


FIG. 20C

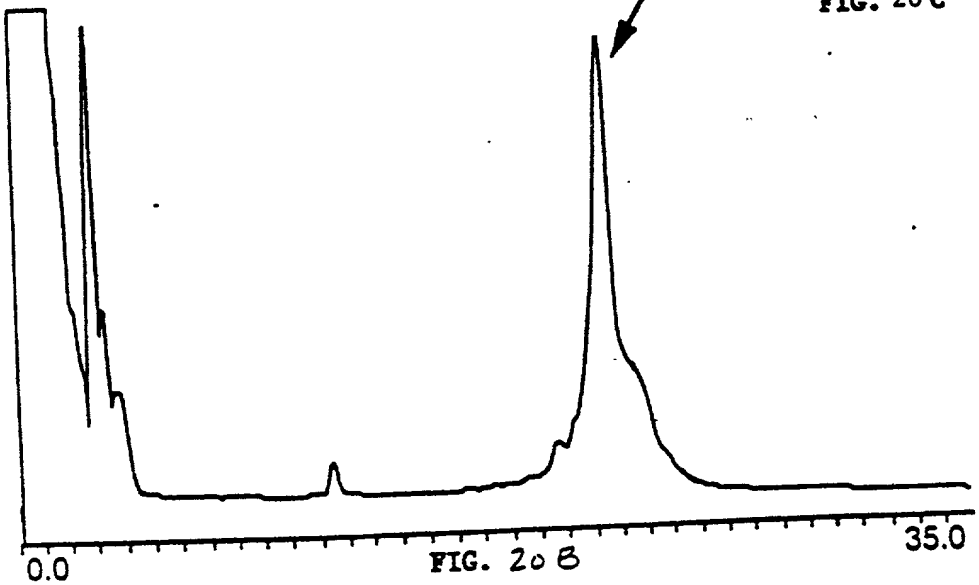


FIG. 20B

Solid Phase Chemical Ligations in the C- to N-terminal Direction

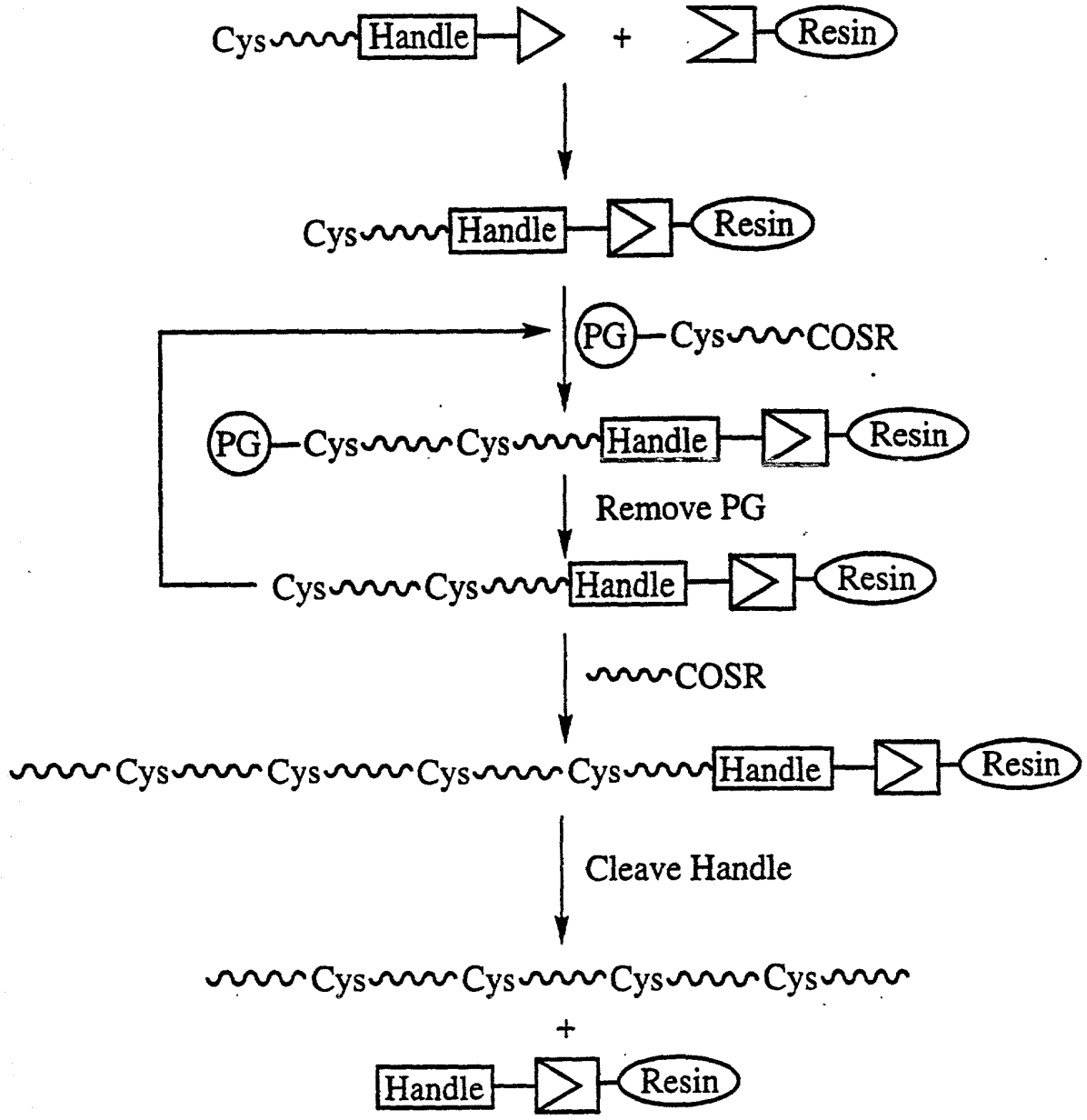


FIG 21

Solid Phase Chemical Ligations in the C- to N-Terminal Direction Synthesis of Phospholipase A2, Group 5 (PLA2G5)

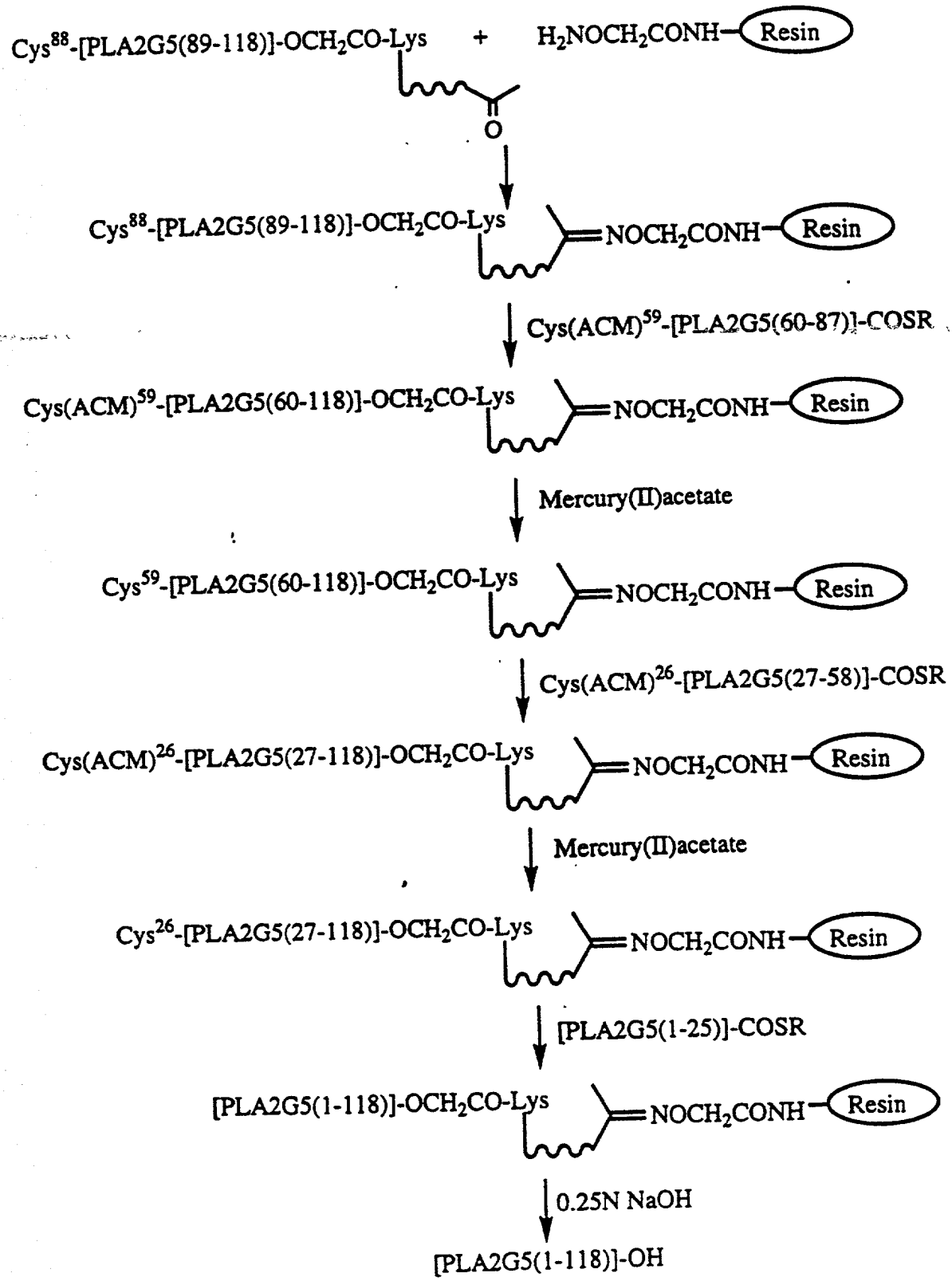


Figure 22

Synthesis of Cam ester derivative

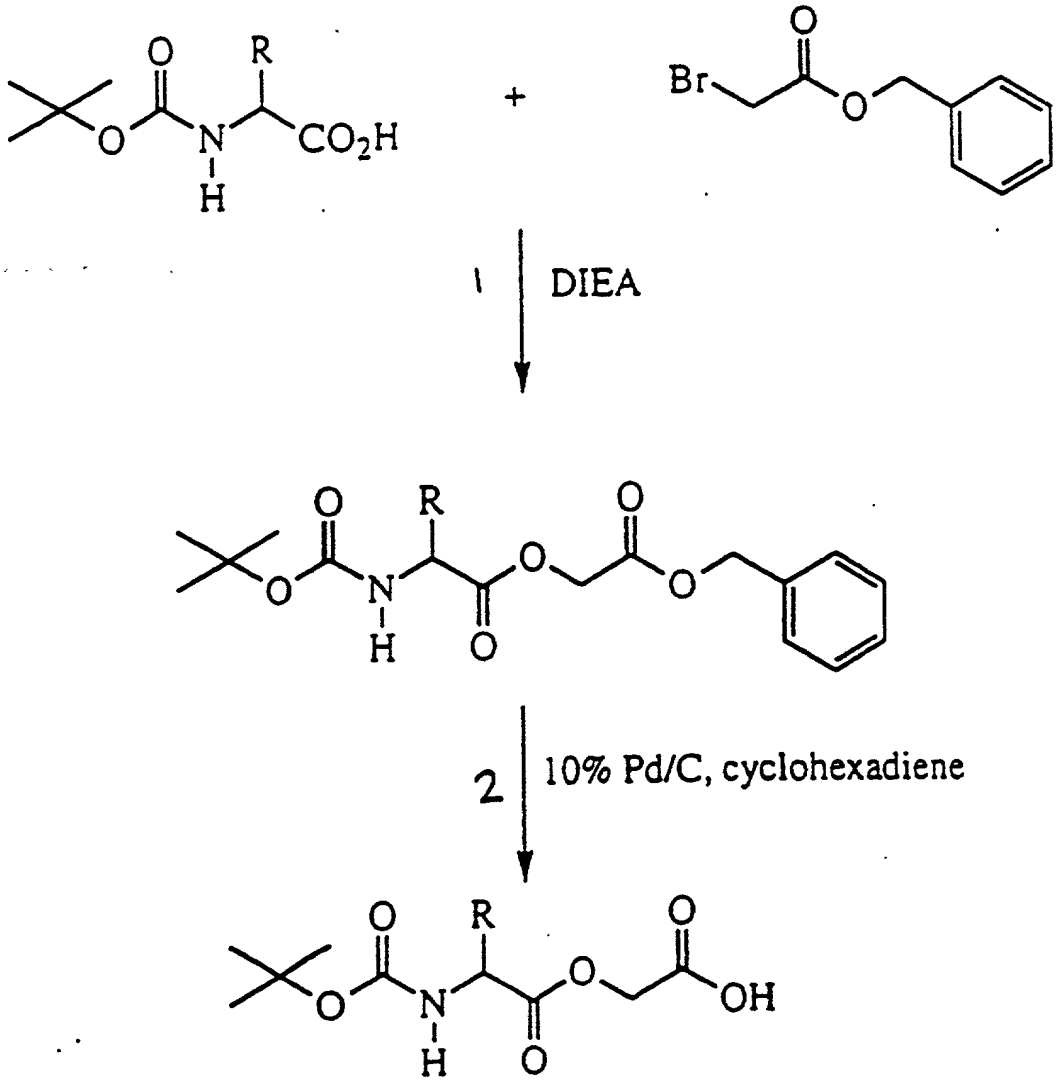


FIG. 23

Synthesis of C-terminal peptide segment

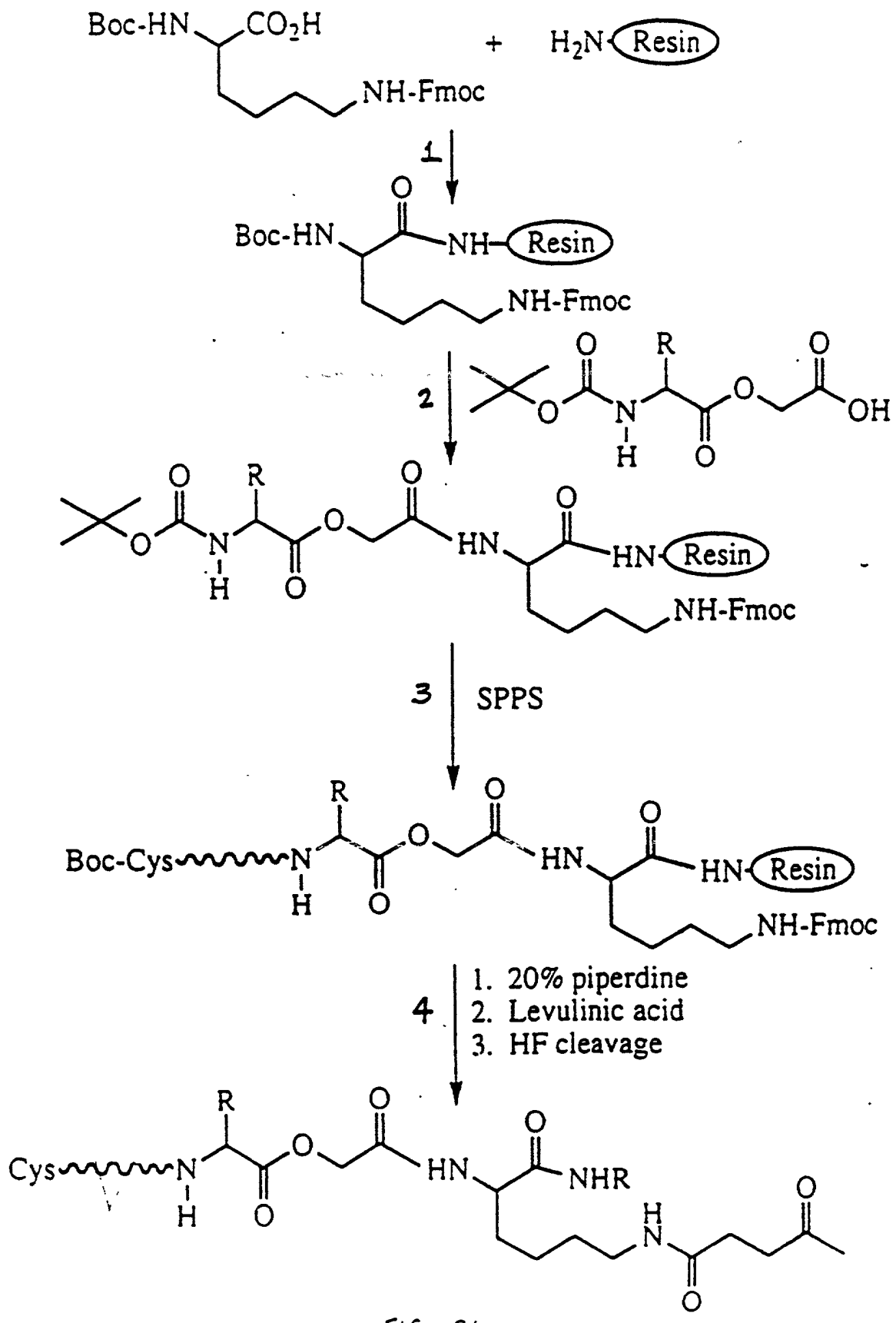


FIG. 24

Universal Solid Phase Chemical Ligation
(Bidirectional Ligations: C- to N-Terminal Ligations First)

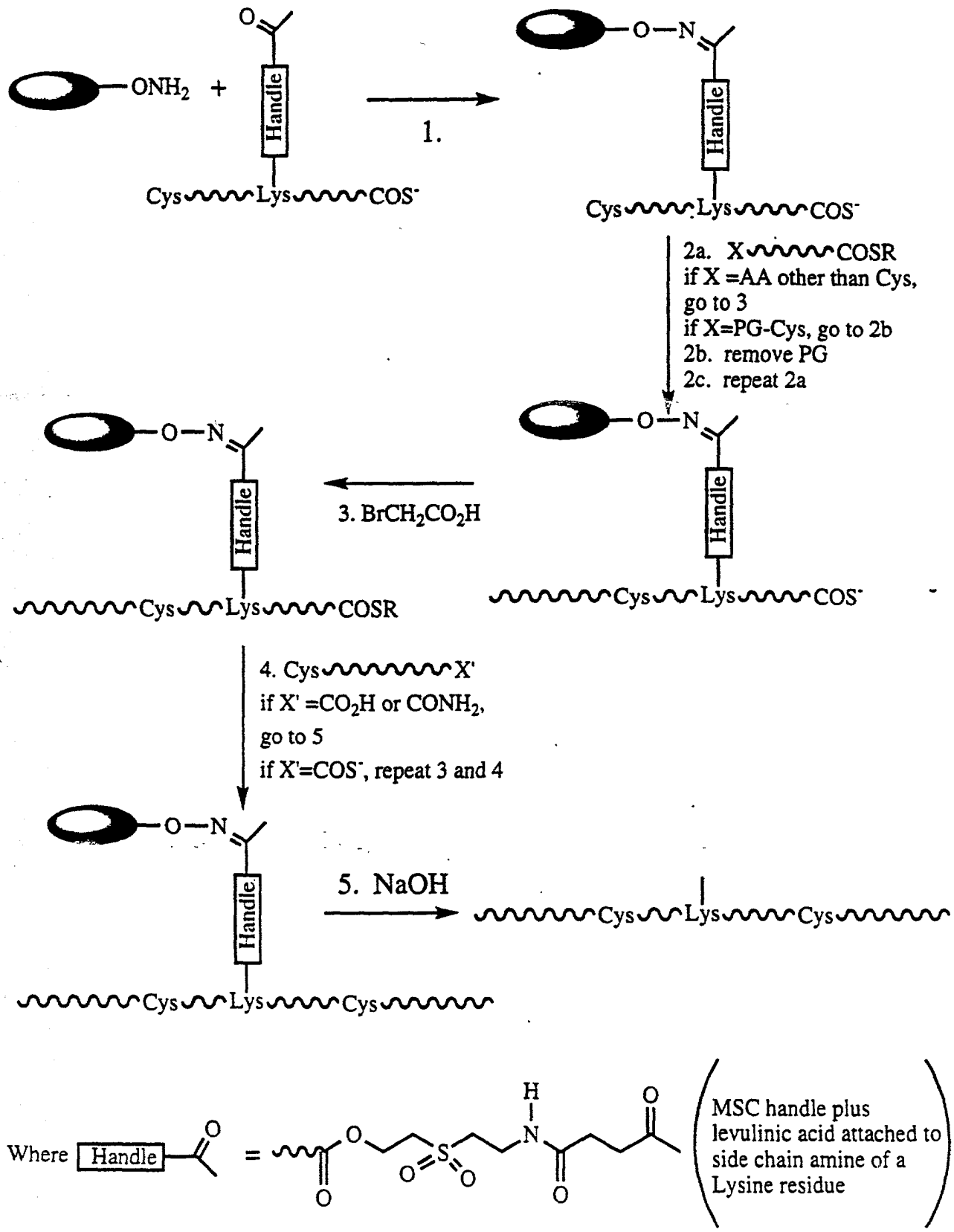


FIG. 25A

Universal Solid Phase Chemical Ligation
(Bidirectional Ligations: N- to C-Terminal Ligations First)

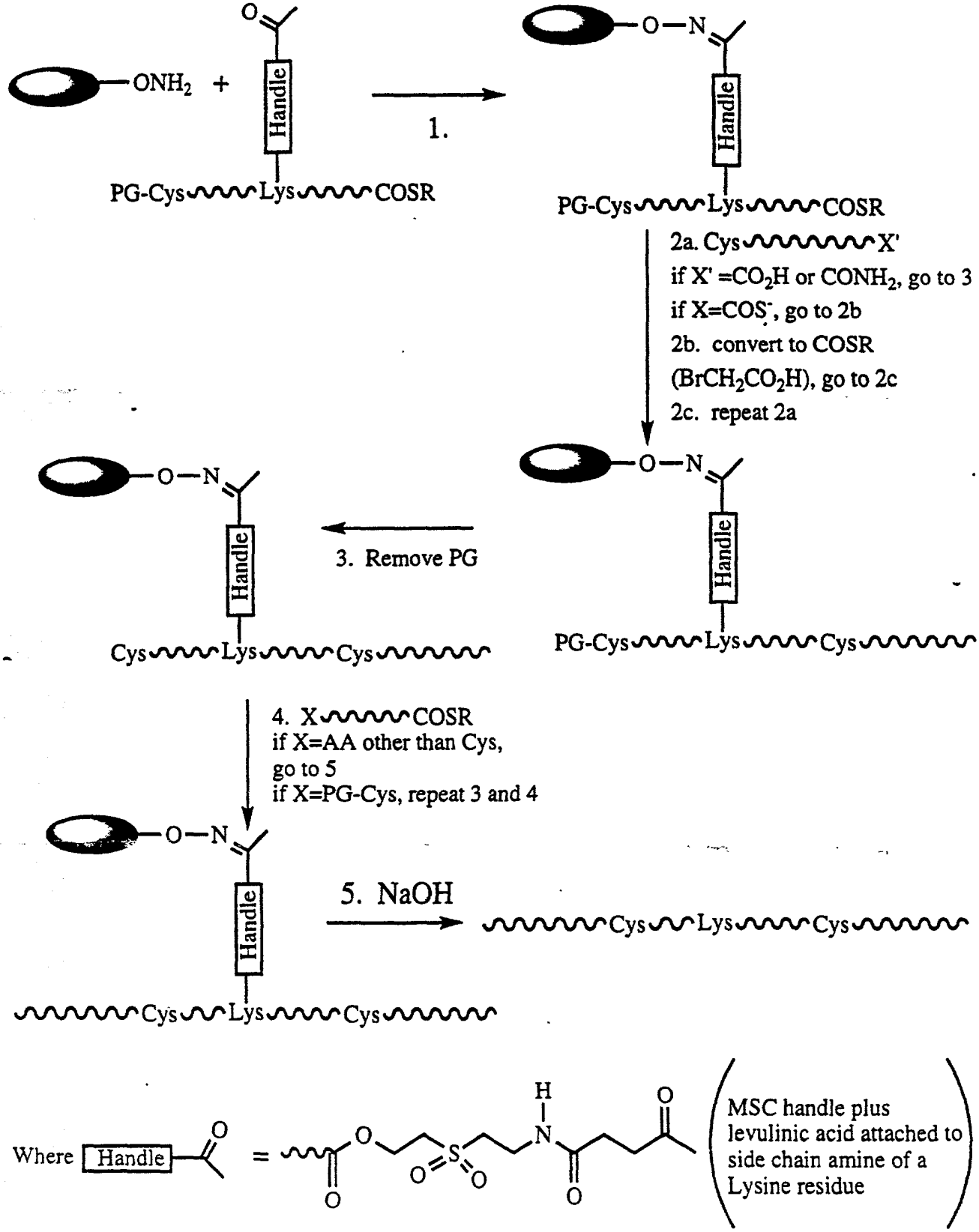


FIG. 25B

Synthesis of Modified Peptide Segment for Universal Solid Phase Chemical Ligation

Starting with an appropriate resin (thioester or thioacid generating), synthesize the peptide using standard Boc protocols until the Lys residue of choice is reached. Couple a Boc-Lys(Fmoc)-OH, then continue the rest of the synthesis.

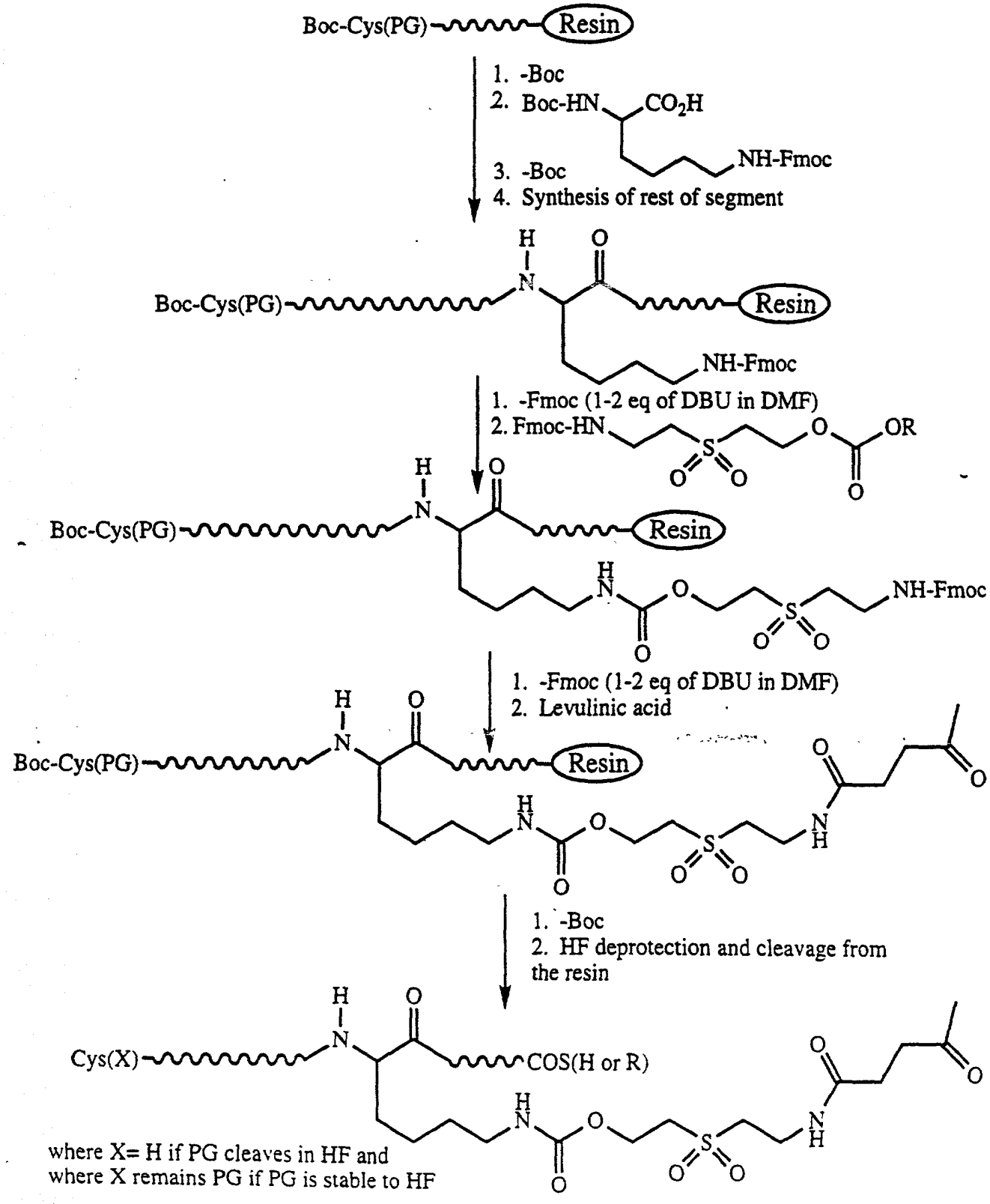
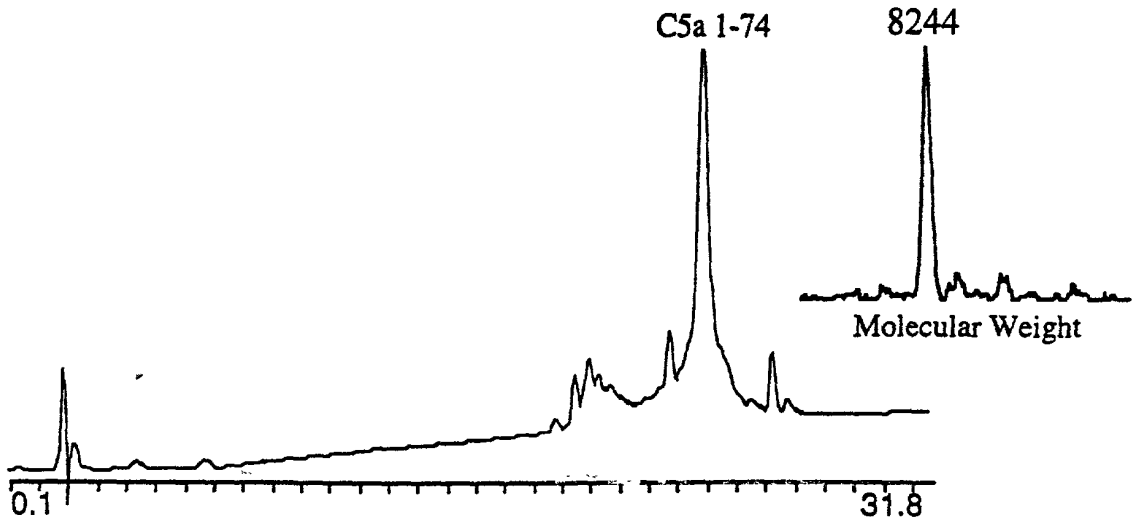


FIG. 25C

Synthesis of C5a by Solid Phase Chemical Ligations in the N- to C-Terminal Direction

1 21 47
TLQKKIEEIAAKYKHSVVKK**CCYDGACVNNDETCEQRAARISLGPK**CIKAFTECC
VVASQLRANISHKDMQLGR
74



Synthesis of C-terminal peptide segment

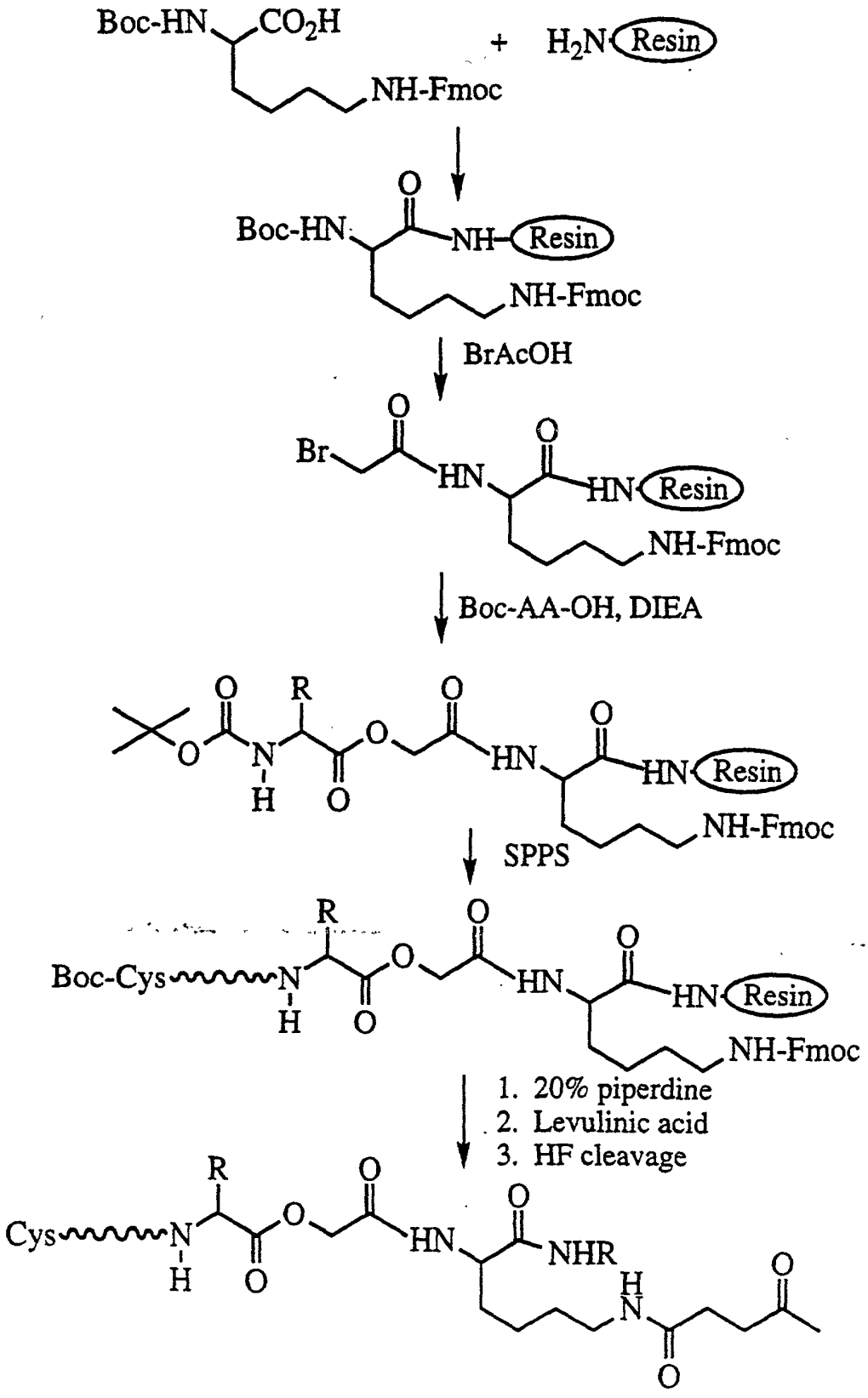


FIG. 27

Synthesis of a Random Sequence by Solid Phase Chemical Ligations in the C- to N-terminal Direction Using Fmoc Protection

ALTKYGFYGCYGRLEEKG**CADRKNILA**
1 10 19 27

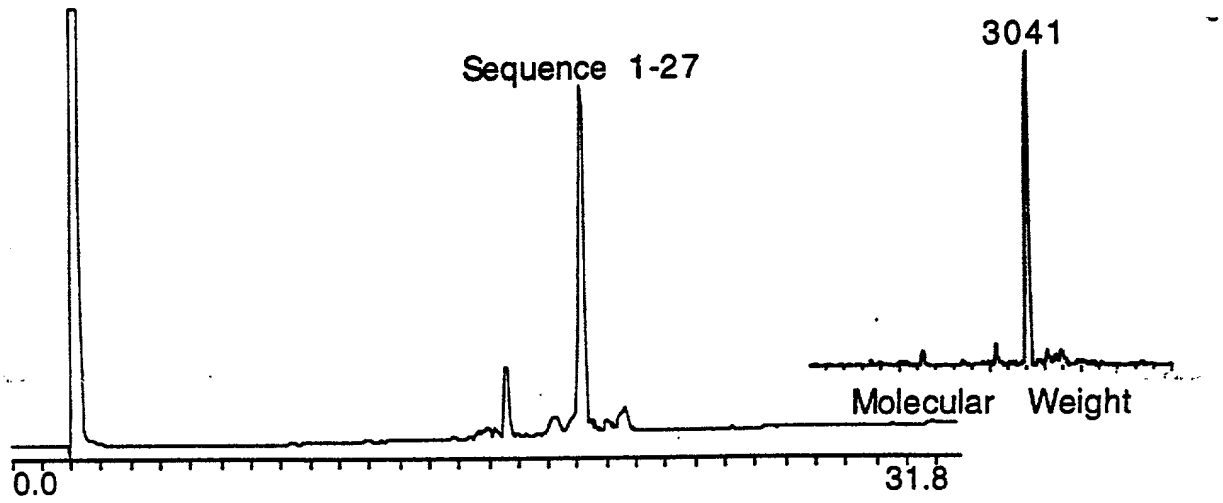


FIG. 28

Synthesis of a Random Sequence by Solid Phase Chemical Ligations in the C- to N-terminal Direction Using ACM Protection

ALTKYGFYGCYGRLEEKG**CADRKNILA**
1 10 19 27

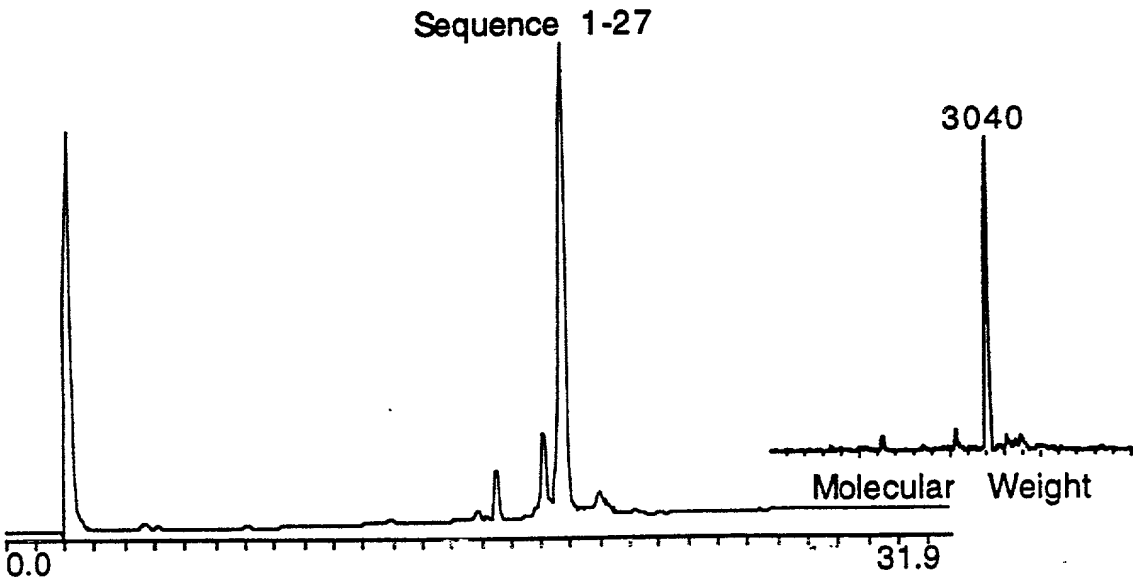


FIG. 29

Synthesis of Phospholipase A2 Group 5 by Solid Phase Chemical Ligations in the C- to N-Terminal Direction

```
1                    26                                    59
GLLDLKSMIEKVTGKNALTNYGFYGCYCGWGGRGTPKDGTDWCCWAHDECYGRLEEKGC
NIRTQSYKYRFAWGVVTCEPGPFCHVNLCACDRKLVYCLKRNLRSYNPQYQYFPNILCS
                     88                                   118
```

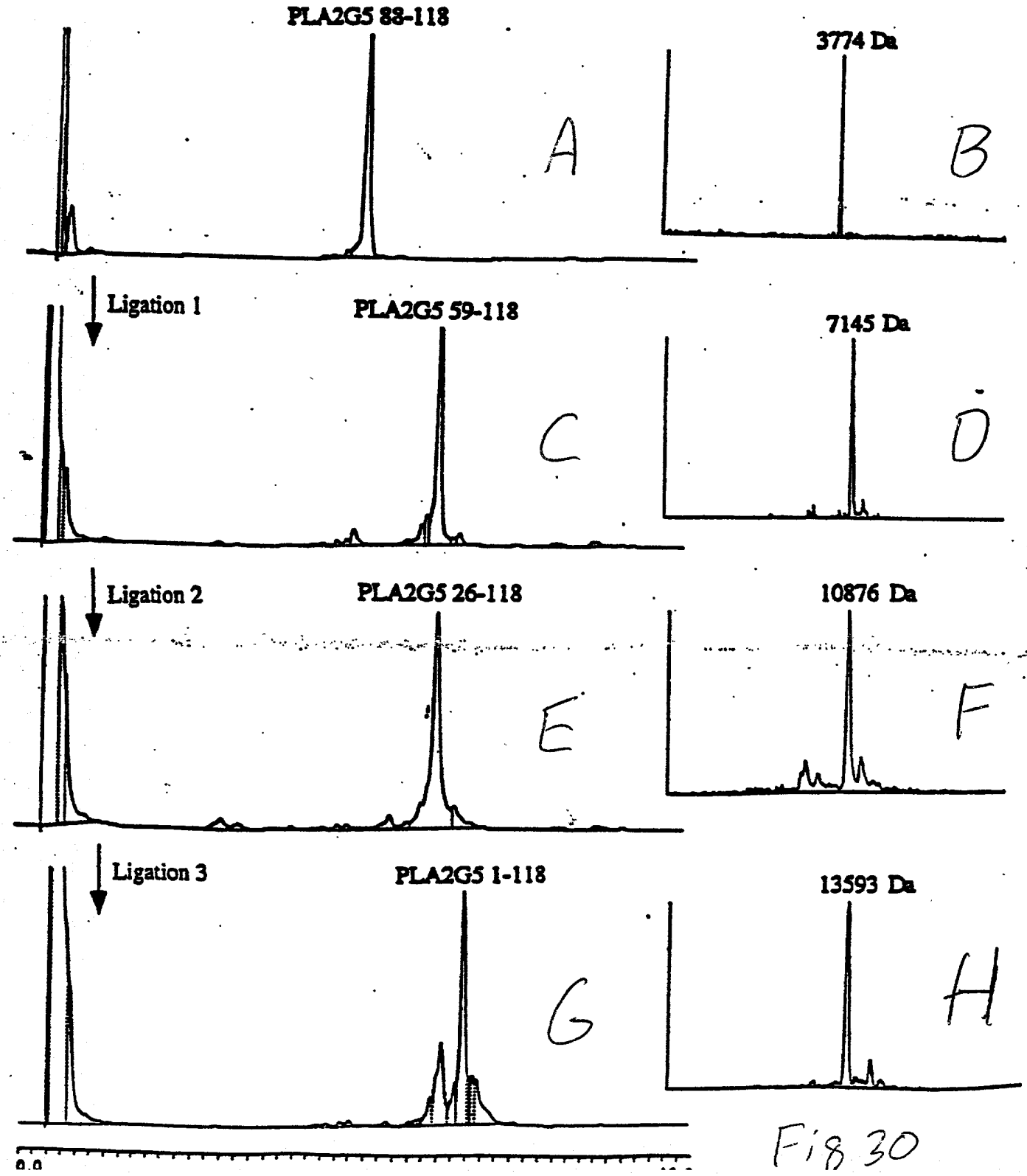


Fig 30

Synthesis of Phospholipase A2 Group 5 by Solid Phase Chemical Ligations in the C- to N-Terminal Direction

1 26 59

GLLDLKSMIEKVTGKNALTNYGFYG**CYCGWGGRGTPKDGTDWCCWAHDHCYGRLEEKGC**

NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRNLRSYNPQYQYFPNILCS**

88 118

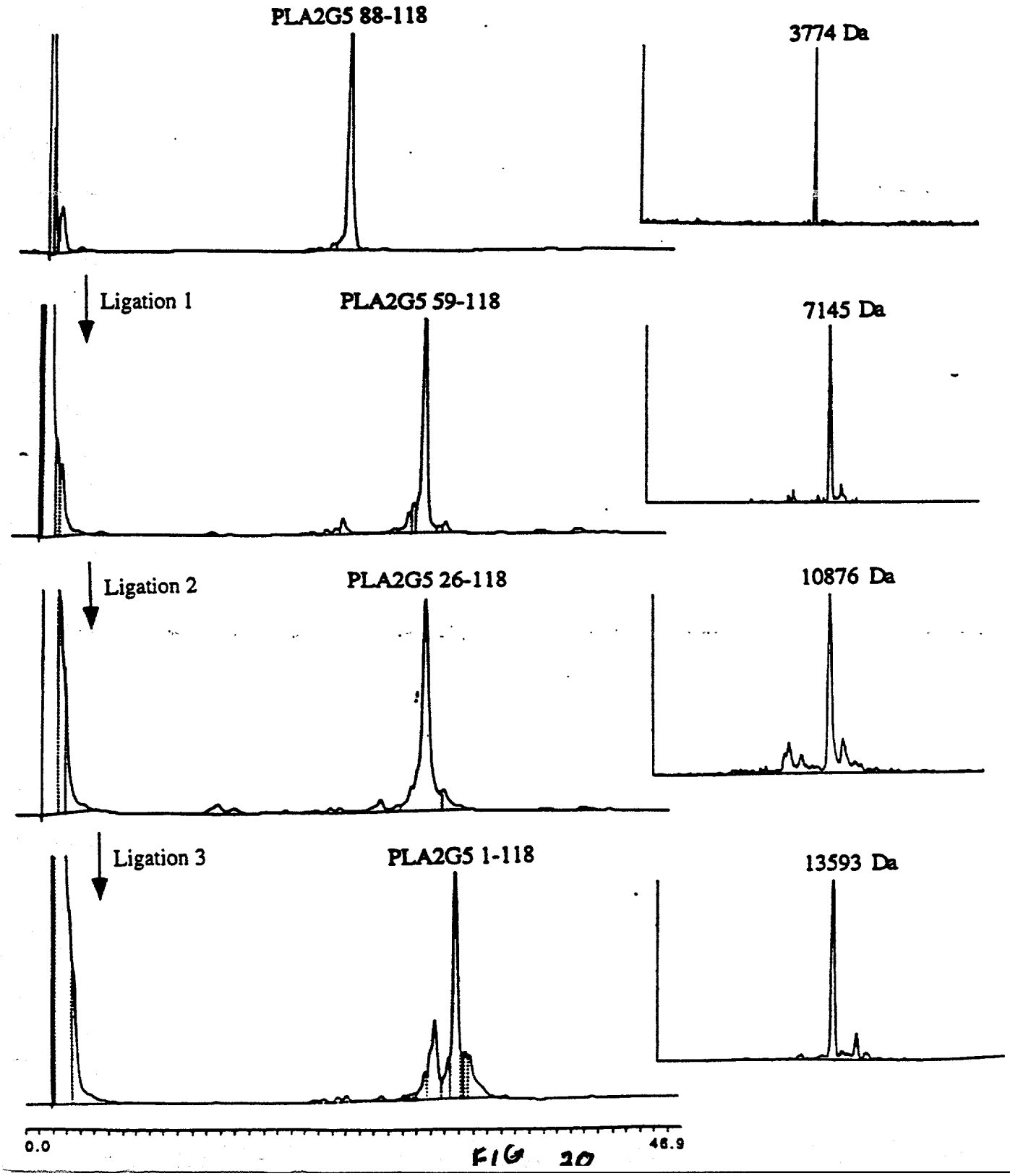


FIG 20